

Aspects on
CEREBRAL VASOSPASM

A clinical and experimental study

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Lund 1981

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by
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"It is with a considerable degree of genuine humility that we broach the topic of cerebral vasospasm. This humility is the result of 20 years of disappointment, frustration, and bewilderment. The feeling of helplessness related to the relentless deterioration of a patient with this entity is matched only by that associated with the care of the victims of spinal cord injury. This is a feeling we have all shared and this has been the primary force driving all of us. It has literally compelled us to turn to the laboratory for answers, and there, in spite of heroic efforts, few revelations and no miracles have emerged."
(Thoralf Sundt & Dudley Davis, the Mayo Clinic, 1979.)



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The present thesis is based on the following papers, which will be referred to by their roman numerals:

- I. Ljunggren B., Brandt L., Kågström E., and Sundbärg G. (1981)
Results of early operations for ruptured aneurysms.
J. Neurosurg. 54:473-479
- II. Brandt L., Ljunggren B., Andersson K-E., Hindfelt B., and Teasdale G. (1981)
Vasoconstrictive effects of human post-hemorrhagic cerebrospinal fluid on cat pial arterioles in situ.
J. Neurosurg. 54:351-356
- III. Boullin DJ., Brandt L., Ljunggren B., and Tagari P. (1981)
Vasoconstrictor activity in cerebrospinal fluid from patients subjected to early surgery for ruptured intracranial aneurysms.
J. Neurosurg. (in press).
- IV. Brandt L., Ljunggren B., Andersson K-E., and Hindfelt B. (1981)
Individual variations in response of human cerebral arterioles to vasoactive substances, human plasma, and cerebrospinal fluid from patients with aneurysmal subarachnoid hemorrhage.
J. Neurosurg. (in press).
- V. Brandt L., Ljunggren B., Andersson K-E., Hindfelt B., and Uski T. (1981)
Effects of indomethacin and prostacyclin on isolated human pial arteries contracted by cerebrospinal fluid from patients with aneurysmal subarachnoid hemorrhage.
J. Neurosurg. (submitted for publication).
- VI. Brandt L., Andersson K-E., Edvinsson L., and Ljunggren B. (1981)
Effects of extracellular calcium and calcium antagonists on contractile responses of human and mesenteric arteries.
J. CBF and Metabol. (submitted for publication).
- VII. Brandt L., Ljunggren B., Andersson K-E., Edvinsson L., MacKenzie ET., Tamura A., and Teasdale G. (1981)
Effects on feline cortical pial microvasculature of topical application of a calcium antagonist (Nifedipine) under normal conditions and in focal ischemia.
J. CBF and Metabol. (submitted for publication).

INTRODUCTION

In 1893 William Gowers concluded that "If the diagnosis of an intracranial aneurysm is certain, the prognosis is extremely grave."¹ This statement is unfortunately still valid.

When in 1920 the British neurologist, Sir Charles Symonds, for some months worked as a voluntary assistant with Harvey Cushing at the Peter Bent Brigham Hospital in Boston, he suggested the diagnosis of a suprasellar aneurysm in a patient whom Cushing was about to operate on. Cushing scoffed at the suggestion. An uncontrollable hemorrhage terminated the operation.²

It happened that the only time Cushing could attend the autopsy was the following afternoon, on which Symonds and the resident, and, as it turned out later, Cushing himself, had tickets for a critical world series baseball game. Cushing decided that the autopsy should be held at that time anyway, and that they all must be present, which they were. When the aneurysm was disclosed Cushing turned to Symonds: "Symonds, you made the correct diagnosis; either it was a fluke, or there was a reason for it. If so, you will prove it. You will cease your ward duties as from now and spend all your time in the library." The resulting paper, giving the first account of the significance of subarachnoid hemorrhage in the diagnosis of aneurysm, appeared in 1923.³ Symond's correct conclusion and Cushing's initiative involved a break-through for clinical diagnosis of intracranial aneurysms.

The first deliberate intracranial attack on a ruptured intracranial aneurysm was carried out by Dott in 1931.⁴ On March 23rd 1937 Walter Dandy performed the first successful operation of an intracranial aneurysm.⁵ He subsequently first reported on a series of operated aneurysm patients in 1944.⁶ During the next decades, however, little evidence arose favouring surgical treatment as compared to the natural history of the disease. It gradually became clear that many patients did not die from rebleeding but from ischemic complications related to the hemorrhage and the frequent occurrence of co-existing intracranial arterial spasm became recognized. Even if the pathophysiological significance of cerebral arterial spasm has been questioned as late as 1975,⁷ most investigators agree with the concept that the presence of spasm to such an extent that cerebral perfusion is affected is important in determining the clinical outcome.

The Swedish pioneers, Herbert Olivecrona and Gösta Norlén, in 1953 con-

cluded that surgery should not be performed until the third week after the aneurysm rupture.⁸ However, by that time approximately half of the patients have died or become severely disabled by recurrent hemorrhages and complicating cerebral ischemic dysfunction. Towards the end of the 1950:ies the aneurysm surgeon, Wylie McKissock in London even stopped operating on ruptured intracranial aneurysms supporting the idea that surgery at any time be not superior to the natural course of the disease.⁹

In 1969 Hamby reviewed the technical aspects of improvements in aneurysm exposure as they had evolved in the previous half century.¹⁰ Pool in 1965 first published on the use of the operating microscope in intracranial aneurysm surgery.¹¹ Yasargil advanced the state of the art of microtechnique for aneurysm surgery, reporting a remarkable drop in mortality rates.¹²⁻¹³ Use of the operating microscope or its equivalent subsequently has become standard technique in surgery of intracranial aneurysms.¹⁴

Despite the common occurrence of cerebral vasospasm in conjunction with aneurysmal subarachnoid hemorrhage it was not until 1965 that the time-course of this phenomenon was elucidated by Kågström et al¹⁵ demonstrating that vasospasm visualized by angiography does not occur until some days after the bleed. This was a further support for a relationship between angiographic vasospasm and delayed cerebral ischemic dysfunction. However, several reports on this subject have not been able to establish an absolute correlation between delayed ischemic dysfunction(DID) and angiographic spasm. As angiography does not demonstrate caliber changes in the resistance vessels it has been suggested that delayed ischemic dysfunction without an angiographic correlate is due to arteriolar narrowing. The fact that even pronounced angiographic vasospasm may occur without concomittant ischemic deficits has been explained by Simeone et al,¹⁶ who showed that in experimental subarachnoid hemorrhage the cerebral blood flow is not consistently reduced until the arteries have become constricted to about one half of their control lumen. According to these findings, reduction in cerebral arterial lumen by vascular spasm does not cause ischemia, unless the arterial diameter is critically reduced, provided that other compensatory mechanisms are not present.

What is the cause of cerebral vasospasm? Already in 1839, John Hunter considered constriction of arteries to be one of the physiological mechanisms responsible for the control of hemorrhage. From later work it is well known that mechanical, chemical and electrical stimulation may induce arterial constriction. Even such nonspecific agents as room air or destil-

led water are known to induce cerebral vasoconstriction.¹⁷

During the last two decades an immense number of studies have been undertaken to elucidate the pathophysiology of cerebral vasospasm and delayed ischemic dysfunction after aneurysmal subarachnoid hemorrhage, but they "have produced more questions than answers" (Sundt & Davis, 1979).¹⁸

The significance of the various mechanisms possibly involved in the phenomenon remains controversial. A large amount of different substances have been suggested to account for the pathogenesis of delayed cerebral hypoperfusion and the list has grown as new vasoactive agents have been discovered. The fact, that vasospasm is not an obligate consequence of aneurysmal subarachnoid hemorrhage, may indicate that variations exist between individuals as regards cerebrovascular responses to chronic exposure for blood-contaminated cerebrospinal fluid.

When shall the sword of Damocles, hanging over the head of the patient with a ruptured intracranial aneurysm, be removed? The last decade's great improvements of surgical techniques and refinement of neuroanaesthesia have markedly improved the results of surgery.¹⁹⁻²⁵ However, the timing of surgery remains controversial,²⁶ although it appears that most neurosurgeons still favour delayed operation as the best method of reducing post-operative ischemic complications. Very early surgery is, however, increasingly proposed with a view to improving the overall outcome not only by preventing rebleeding but also of preventing delayed ischemic dysfunction by operative removal of perivascular blood collections.^{19,21,27}

Several studies have confirmed the presence of vasoconstrictor material in post-hemorrhagic cerebrospinal fluid (CSF) from patients suffering from aneurysmal subarachnoid hemorrhage (SAH). So far no complete information about the identities of the vasoconstrictor substances in the CSF is available. It has been proposed in some earlier studies that the development of vasospasm and delayed cerebral ischemic dysfunction is paralleled by an increase in the concentration of vasoconstrictor agents in the CSF.²⁸⁻²⁹

The presence or absence of cerebral vasoconstriction after aneurysmal SAH implies an interaction between vasoconstrictor substances in the post-hemorrhagic CSF and protective mechanisms in the cerebral arterial wall to maintain normal vascular tone. Most experimental results on vasospasm have been obtained in studies on inbred animals with relatively homogeneous vascular responses. The capricious appearance of vasospasm in some but not all patients with an aneurysmal SAH may be due to variations in the vascular responsiveness. This is supported by clinical experience from early

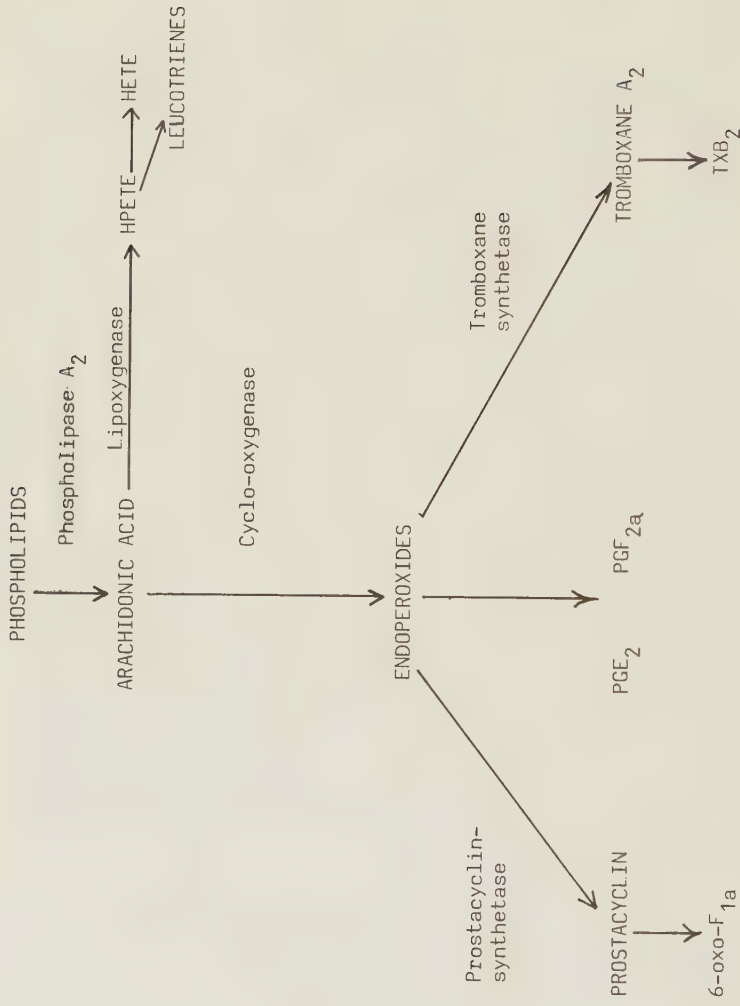


Fig.1 Pathways of arachidonic acid metabolism.

aneurysm surgery: some patients with heavily blood-contaminated subarachnoid spaces in whom a wash-out was not technically feasible, yet, did not develop any indication of cerebral arterial spasm or delayed ischemic dysfunction. This may imply marked individual characteristics in human cerebral arteries and arterioles.

Because of the numerous substances incriminated in vasospasm, a variety of drugs have been tried in an effort to prevent or treat intracranial spasm following SAH. Despite many promising preliminary investigations, the final results have been disappointing. Some of the failures may be ascribed to the fact that the basis for the trials has been derived entirely from experimental animal models. However, there is no animal model of delayed ischemic events that parallels the syndrome of cerebral vasospasm seen in humans. This brings Pope's dictum to the fore: "The proper study of mankind is man".

Lately, evidence has been accumulating suggesting that other mechanisms may be involved in the pathogenesis of cerebral ischemic dysfunction in SAH patients. Among these are lesions in the wall of the spastic artery, including endothelial damage with associated thrombus formation.^{30,31} Based upon the knowledge that vasoactive prostaglandins are synthesized in the endothelium, it has been suggested that cerebral vasospasm may represent insufficient synthesis of dilatatory arachidonic acid metabolites. Experimental studies and studies of cerebral arteries from patients who have died from delayed cerebral dysfunction after SAH have demonstrated marked morphological changes within the vessel wall paralleling the onset of vasospasm. Elevated levels of lipid peroxides have also been implicated in vasospasm, and it has been speculated that vasospasm may be the result of an interaction between accumulated hydroperoxides, derived from the lysed blood clot, and a modified synthesis of prostaglandins. This should provide a predominance for vasoconstrictor metabolites.³²⁻³⁵ (Fig.1)

The failure of prior attempts to treat cerebral vasospasm with various agents, utilizing different mechanisms, suggests that a reasonable approach to the problem would be interfering with the final step in the smooth muscle contraction mechanism. It is generally accepted that changes in the concentration of calcium ions within the smooth muscle cell regulate several cellular processes, including the final step in the excitation-contraction coupling. Several studies have shown that smooth muscle is dependent on an extracellular source of calcium to initiate and maintain contraction. Intracellularly stored calcium is also involved, but is of less importance.

Many pharmacological agents blocking transmembrane calcium-flux are used clinically, especially in cardiovascular disorders. Many substances are classified as "calcium antagonists", but only a few of them are considered selective influx-blockers. However, contradictory information about the exact site of action have been presented even for these drugs as well as indications of regional and species differences. Most information of the effects of calcium-inhibiting drugs have been obtained from animal studies on the systemic circulation. There is little information about the cerebro-vascular effects, especially in humans.

THE MAIN OBJECTIVES OF THE PRESENT STUDY WERE:

To analyse the results of early surgery (i.e. within 60 hrs after aneurysm rupture), combined with attempts at peroperative removal of subarachnoid clots and blood-contaminated cerebrospinal fluid, and to evaluate the effects of such early surgery with special regard to the incidence of DID (Paper I).

To investigate whether perivascular application in vivo of posthemorrhagic cerebrospinal fluid from patients with aneurysmal subarachnoid hemorrhage induces constriction of capillary arterioles and resistance vessels, i.e. vessels not visualized on angiography, and whether the calcium-antagonist nifedipine counteracts constriction induced by post-hemorrhagic cerebrospinal fluid (Paper II).

To investigate the vasoconstrictor activity in cerebrospinal fluid from early surgery patients (see above) on a daily basis and to relate the activity to the appearance of vasospasm and ischemic dysfunction (Paper III).

To investigate whether there are any individual differences in the responses of human pial arteries to vasoactive substances, human plasma and post-hemorrhagic cerebrospinal fluid which should possibly account for the capricious clinical appearance of cerebral vasospasm (Paper IV).

To investigate whether an impaired arterial prostaglandin synthesis modifies the vasoconstrictor effect of post-hemorrhagic cerebrospinal fluid on human cerebral arteries. Does prostacyclin and its metabolite 6-keto-PGE₁ counteract contractions of human pial arteries exposed to post-hemorrhagic cerebrospinal fluid? (Paper V).

To evaluate the importance of extracellular calcium for the contractile ability of human pial arteries and to study the relaxant effects of the calcium-influx inhibiting drugs nifedipine and nimodipine. Does the relaxant effect depend on in which way constriction is induced? Do vessels from different vascular regions respond differently? (Paper VI).

To study the in vivo effects of locally applied nifedipine on the cerebrovascular bed, arteriolar versus venular responses, and the effects in focal ischemia (Paper VII).

METHODS

The first paper in this study is an analysis of the clinical outcome in a consecutive series of 219 patients with a ruptured aneurysm of the anterior part of the circle of Willis, referred to the neurosurgical clinic in Lund during a 4-year period. Early intracranial operation (within 60 hours after SAH) was performed in 81 patients whilst 55 patients had surgery 8 days or more after aneurysm rupture and 53 patients were not operated upon. The operations were performed via the pterional approach described by Yasargil and Fox.¹⁵ The basal cisterns were opened and attempts were made at removal of subarachnoid clots and rinsing the basal cisterns from blood-contaminated cerebrospinal fluid.

Results were determined at follow-up examination 6 months to 4 years after surgery. Clinical outcome was classified as good, fair or poor recovery. Outcome was compared between patients undergoing early versus conventional later surgery. The outcome for early surgery patients was also compared to the outcome for the patients who fulfilled the criteria for, but did not undergo early surgery. The incidence of delayed ischemic dysfunction was determined. In search for complicating factors, age and sex distribution, as well as aneurysm location and arterial hypertension prior to SAH were taken in account.

In paper II the vasoconstrictive effects of human post-hemorrhagic cerebrospinal fluid was studied on cat pial arterioles in situ. The parietal cortex was exposed in anesthetized cats. Periarteriolar microinjections of CSF from SAH patients were performed at normocapnia and normotension. Arteriolar caliber changes were studied using a stereo-microscope with an image-splitting device. Post-hemorrhagic CSF from four patients suffering from SAH due to a ruptured aneurysm was used. Three of the patients had severe delayed neurological deficits and one patient had pronounced angiographic vasospasm but remained free from neurological symptoms. The responses to patient CSF were compared as well for individual differences as versus the response to control and mock CSF. The importance of resting arteriolar caliber for the responses

was evaluated. Potassium concentration as well as pH of the CSF specimens were determined and correlated to the vasoconstrictor activity. Finally the effect of nifedipine on vasoconstriction induced by patient CSF was investigated.

In paper III, CSF vasoconstrictor activity was examined in serial samples from 10 patients undergoing early aneurysm surgery. The patients were 7 females and 3 males. All patients were neurological Grade III¹⁶ or less prior to surgery. CSF from the patients was collected either by lumbar puncture or from the lateral ventricle. In 4 cases cisternal CSF was collected during surgery by placing a catheter with its tip into the opened basal cisterns prior to aneurysm dissection and clipping. CSF samples were collected up to 18 days post-SAH. All CSF was immediately frozen at -20°C and samples remained frozen until assayed for vasoconstrictor activity. In an organ bath 0,5 ml aliquots of CSF were bioassayed on either a rat stomach fundus, or human isolated basilar or vertebral arterial preparations. Contractions were recorded isotonicly and responses to CSF were quantitated by comparison with contractions induced by prostaglandin PGE_2 . In an attempt to evaluate the identity of vasoconstrictor substances in CSF, different pharmacological blocking agents were tested with the aim of reversing contractions induced by CSF. Potassium was measured in each CSF sample by flame emission photometry. Vasoconstrictor activity was compared to the neurological state and presence or absence of vasospasm at postoperative angiography.

The capricious appearance of delayed cerebral vasospasm in some but not all patients with an aneurysmal SAH was the incitement for paper IV. An in vitro study was undertaken with the aim of evaluating possible individual differences in the response of human pial arteries to vasoactive substances and post-hemorrhagic CSF. Small cortical arteries were removed from macroscopically normal parts of the human brain in conjunction with lobe resection for a deeper localized glioma. Usually, within 10-15 min the vessel segments were mounted in an organ bath. After equilibration with a passive load of approximately 5 mN, the arteries were exposed to different vaso-

active substances including CSF from patients with aneurysmal SAH. Isometric contractions were recorded by means of Grass FT 03C transducers. Vascular responses were expressed as per cent of contraction induced by 127 mM potassium. Preparations from 22 patients were investigated, and the responses were related with regard to age, sex, tumor location and medical treatment at the time of surgery.

Paper V deals with the question whether an impaired prostaglandin synthesis may affect the vasoconstrictor activity of post-hemorrhagic CSF on human pial arteries. To that end, the vascular responses to post-hemorrhagic CSF were studied before and after pretreatment with the cyclo-oxygenase inhibitor indomethacin. As it has been speculated that cerebrovascular spasm may arise from an impaired synthesis of prostacyclin and/or other vasodilating metabolites from arachidonic acid, it was of interest to evaluate the effect of prostacyclin on CSF-induced contractions of human cerebral arteries. For this purpose, prostacyclin as well as its stable metabolite 6-keto-PGE₁ were investigated for their effects on CSF-induced contractions and contractions induced by potassium, noradrenaline, 5-hydroxytryptamine and prostaglandin F_{2a}. The in vitro model used for this study was identical with that described in paper IV.

Paper VI concerns the importance of extracellular calcium for contractions of human pial arteries and is a study of the effects of the two calcium antagonists nifedipine and nimodipine on human pial and mesenteric arteries contracted by potassium, noradrenaline, 5-hydroxytryptamine and prostaglandin F_{2a}. The in vitro model used was identical with that described in paper IV. The ability of the calcium antagonists to relax preparations contracted by potassium (127 mM) or PGF_{2a} (2.5 mM) were evaluated. The calcium antagonists were added cumulatively, and their relaxant effects were expressed as per cent of the induced contraction. Also, the inhibitory effect of the drugs on contractions induced by noradrenaline and 5-hydroxytryptamin were investigated. In experiments performed to investigate the importance of extracellular calcium for the effects of contractile and relaxant agents the vascular re-

sponses were studied after a standard 'pretreatment' in a calcium-free medium for 30 minutes.

Paper VII is a study of the effects of topical microapplication of nifedipine on the cat cortical pial microvasculature under normal conditions and in focal ischemia. The experimental model used for perivascular microinjections and pial vessel caliber measurement was identical with that described in Part III. Seventeen cats of either sex were used and the experiments performed under steady state conditions. Nifedipine in five different concentrations was applied to the arterioles whereas the venules were exposed to one and the same concentration. Test solutions containing the nifedipine vehicle corresponding to the different concentrations were also used. Focal ischemia in the parietal cortex was obtained by clamping the left middle cerebral artery (MCA) through a transorbital exposure. Diameter changes were expressed as percentage deviation from resting vessel caliber.

RESULTS

The clinical study (Paper I) is focused on the results in the consecutive series of 81 patients who had early intracranial operation (within 60 hours after SAH) combined with attempts to remove as much subarachnoid clots and blood-contaminated CSF as possible from the basal cisterns. 60 patients (74%) made a good recovery. Eight patients died within a month. Five patients were severely disabled and died within 2-8 months.

In a total of 17 patients (21%) although the immediate post-operative course was uneventful, evidence of cerebral ischemic dysfunction developed 4-13 days after the bleed and resulted in death in 8 patients. Delayed ischemic neurological deficit may occur in 26-36 per cent of patients with aneurysmal SAH.^{17,18} The present results do not indicate that the capricious appearance of delayed cerebral ischemic dysfunction can be dramatically reduced by our present operative procedures. Among patients with arterial hypertension prior to SAH, 50 per cent developed delayed ischemic dysfunction in contrast to 13 per cent of the normotensive patients.

Recently¹⁹, it was stated that patients with angiographically pronounced vasospasm eventually showed neurological symptoms. The observations in the present series are not in agreement with this statement. Some patients developed severe angiographical spasm but remained free from symptoms. On the other hand, some patients with symptoms of severe delayed ischemia demonstrated only a fairly moderate narrowing of the larger cerebral arteries on angiography. These observations stress the importance of the distribution of vasospasm and the adequacy of the patient's compensatory mechanisms. Evidently, spasm in the conducting arteries is only one factor that determines cerebral perfusion after SAH.

In paper II the vascular effects of CSF from four SAH patients were studied on 36 individual cat pial arterioles. With a single exception, the CSF's caused a marked constriction of the arterioles, the maximum response occurring 1 to 4 minutes after the perivascular microinjection. The extent of induced vasoconstriction increased significantly with decreasing vessel size. There were no significant differences in constricting activity between the four specimens obtained from 3 patients with pronounced delayed ischemic dysfunction and from one patient without symptoms.

In 24 experiments the effect of an additional perivascular application of nifedipine was tested when the CSF-induced vasoconstriction had reached a plateau. A marked dilatation was consistently seen and its extent varied inversely with resting vessel caliber. The duration of dilatation was however, shorter in arterioles previously exposed to post-hemorrhagic CSF as compared to normal vessels of comparable size.

The CSF-induced constrictions were of much longer duration than those observed after microapplication of 5-hydroxytryptamine, not favouring this amine being a major contributor to CSF-induced vasoconstriction.

In paper III it is shown that the vasoconstrictor activity in CSF from 10 early operated patients was generally low compared to previous studies of late surgery patients. There was no obvious difference in vasoconstrictor activity between pa-

tients who developed delayed ischemic deficits versus patients who remained free from symptoms. Generally, vasoconstrictor activity was high shortly after SAH, and then declined. Clinical deterioration was not paralleled by increased vasoconstrictor activity. In fact, the last values for vasoconstrictor activity (obtained 4 days prior to death) were low in two patients who died in progressive cerebral ischemia.

CSF potassium concentrations were in the same range in the 10 patients (1-5 mmol/l) and never exceeded 10 mmol/l). The highest potassium concentration in any CSF sample (9 mmol/l) induced only a very small contractile response, equivalent to 7.3% of the maximum response produced by CSF. Accordingly, CSF-induced contractions were not caused by K^+ . However, there was a significant correlation between K^+ -concentration and vasoconstrictor activity in the CSF.

In paper IV CSF from 6 patients suffering from an aneurysmal SAH and with pronounced symptoms of delayed ischemic deficits were tested in vitro on human cerebral arteries. All the CSF samples induced constriction in vessels from one or several donors. On the other hand, twelve vessels did not react to any CSF. Although a marked variability in the CSF responses were noted between vessels from different donors, the pattern of individual vascular response to different CSF specimens was rather consistent. With one exception, noradrenaline and 5-hydroxytryptamine induced contractions of vessels from all donors. The responses to the amines varied markedly, not only when compared to those induced by potassium but also between the two amines. Prostaglandin F_{2a} evoked marked contractions of all investigated vessels, in two preparations even exceeding those induced by potassium. Similar to CSF there was a marked variation in the individual responses to human plasma and blood.

In human cerebral arteries, preincubated with indomethacin (Paper V), contractions induced by cerebrospinal fluid from patients with aneurysmal SAH were markedly increased. Also contractions induced by noradrenaline, but not by 5-hydroxy-

tryptamine, were augmented. Prostacyclin and its metabolite 6-keto-PGE₁ invariably reversed contractions induced by cerebrospinal fluid (CSF) as well as noradrenaline and 5-hydroxytryptamine. The relaxant effects were similar on indomethacin-augmented contractions.

6-keto-PGE₁ had an inconsistent and weak relaxant effect on contractions induced by potassium, whereas prostacyclin produced a small but consistent relaxation. In preparations contracted by prostaglandin F_{2a} both prostacyclin and 6-keto-PGE₁ had a pronounced relaxant action.

In the in vitro studies on human arteries (Paper VI), potassium induced concentration dependent contractions. The threshold concentration of potassium eliciting a contractile response differed in mesenteric and cerebral arteries. In cerebral arteries approximately 10 mM potassium induced contraction; in mesenteric arteries a 3 to 5 mM higher concentration was needed. In both types of vessel more than half maximum effect was reached at a concentration of 20 mmol/l. In cerebral arteries PGF_{2a} induced concentration dependent contractions, maximum tension being obtained at a concentration of 2.5 uM. Maximum PGF_{2a}-induced contractions were equal to potassium (127 mM) induced contractions. Mesenteric arteries rarely responded to PGF_{2a} and when a contraction was recorded, it was weak and unstable.

After 30 minutes incubation of the vessels in a calcium-free medium, the responses to potassium, noradrenaline and 5-hydroxytryptamine were markedly reduced. After reintroduction of calcium and incubation for 30 minutes, potassium-induced contractions were completely restored in contrast to noradrenaline and 5-hydroxytryptamine induced contractions.

Nifedipine and nimodipine caused almost complete relaxation of potassium-induced contractions. The same concentration (0,15 uM of nifedipine) reduced contractions produced by noradrenaline to 29 per cent and that of 5-hydroxytryptamine to 42 per cent of the control contractions obtained by the amines in the absence of the calcium-antagonist. Potassium-contracted mesenteric arteries were also almost completely relaxed by the calcium antagonists. Minor differences in EC 50 values between

the two vascular regions were recognized. Nifedipine was more potent on cerebral vessels, but in this respect no statistical difference for nimodipine was established.

Nifedipine and nimodipine, both relaxed PGF_{2a} contracted pial arteries by about 60 per cent; there were no differences in EC_{50} values between the two drugs. However, significantly higher EC_{50} values were observed when compared to effects on potassium induced contractions.

Arterial depolarization by 127 mmol potassium after incubation for 30 minutes in a calcium-free medium depressed the contractions to about 40 per cent of maximum potassium induced contraction in normal buffer solution. In the presence of nifedipine ($0,15 \mu\text{M}$) no contractile effect was obtained.

In paper VII it was shown that topical application of nifedipine caused a marked, concentration dependent arteriolar dilatation in vivo. The dilatatory responses increased significantly with decreasing arteriolar size and were prompt with peak values mostly reached within 90 seconds followed by a slow decline. Resting vessel caliber was reached approximately 15 minutes after the microinjections. With the highest nifedipine concentration used ($40 \mu\text{g/ml}$) mean arteriolar dilatation was almost 90 per cent from resting vessel caliber.

Perivenular microapplication of nifedipine invariably caused dilatations which however, were less pronounced but much more longlasting than on the arteriolar side.

After occlusion of the middle cerebral artery (MCA), all pial arteries demonstrated a dilatation which became apparent 15-20 seconds after vessel occlusion. The arterioles in the antero-lateral gyrus showed sustained dilatation whereas delayed constriction occurred in the suprasylvian gyrus and most frequently in the ectosylvian gyrus 2-9 minutes after MCA-occlusion. Periarteriolar microapplication of nifedipine on constricted 'ischemic' arterioles invariably caused a marked dilatation. When compared to normal vessels the dilatatory responses were more transient. In some occasions where stasis had been present following MCA occlusion, a transient return of flow was observed after microapplication of nifedipine.

GENERAL DISCUSSION

Despite several advances in medical and surgical care of patients suffering from an aneurysmal SAH during the last decades, morbidity and mortality remain high. Patients continue to die or become disabled at unacceptable rates as a consequence of repeat hemorrhages or complications related to the initial bleed, i.e. vasospasm and delayed ischemic dysfunction. In the early period when surgical treatment of intracranial aneurysms was developed, patients were generally operated upon in the acute stage, but mortality and morbidity were unacceptably high. When the frequent occurrence of intracranial arterial spasm was recognized, it seemed logical to assume, that additional perioperative brain retraction, and sometimes induced systemic hypotension, could cause further cerebral hypoperfusion resulting in infarction. With this background, most neurosurgeons prefer delayed operation for ruptured intracranial aneurysms.

During the last decade mortality rate has dropped markedly. This improvement, however, can not only be ascribed to changes in operative timing, because during the same period microsurgical techniques and other improvements were introduced to aneurysm surgery. Even if delayed surgery is safer as an operative procedure, repeat hemorrhages and/or complicating ischemic dysfunction during the waiting period contribute to the prevailing high management morbidity and mortality.

Though most surgeons still favour delayed operation, early surgery is increasingly proposed not only with a view to improve the overall outcome by preventing rebleeding but also in an attempt to prevent ischemic complications by removal of subarachnoidal blood. The latter concept is based on the observation of a relationship between the amount of blood in the subarachnoidal space and the subsequent development of vasospasm and delayed ischemic cerebral dysfunction.^{36,37}

So far there is no randomized study to document the outcome of patients undergoing early aneurysm surgery versus the outcome with delayed treatment. The uncertainty in this matter is reflected in a comprehensive review from the Mayo Clinic (1978),³⁸ in which the authors were unable to recommend an ideal time for surgical intervention. Even if the present series is not randomized, it permits a comparison of outcome between early surgery versus delayed treatment.

In paper I, a comparison was made between the outcome for patients receiving early surgery versus the outcome for those patients who would have fulfilled our criteria for such surgery, but who were not operated early. The comparison indicates that early surgery resulting in 74 % good recoveries, 10 % morbidity and 16 % mortality is superior to delayed treatment, which resulted in 56 % good recoveries, 18 % morbidity and 26 % mortality. In fact, when considering good recoveries only, the results of early surgery were fully comparable to the results in the selected group of patients subjected to conventional delayed surgery (74 % versus 76 % good recoveries).

The incidence of delayed ischemic neurological deficits ranges from 26 - 36 per cent in patients with SAH.^{39,40} The 21 % incidence in our series of early operated patients, indicates that our present operative procedures do not eliminate but may reduce the incidence of delayed ischemic dysfunction. If the three patients are excluded, in whom a peroperative cistern cleaning could not be performed for various reasons (see Paper I), the incidence of delayed ischemic dysfunction was 18 % (14 of 78 patients).

Several studies indicate that arteries in hypertensive individuals differ morphologically from those of normotensive individuals.^{41,42} Experimentally, spontaneously hypertensive animals are more vulnerable than normotensive to carotid ligation, indicating reduced compensatory mechanisms. Consequently, the poorer outcome for hypertensive patients in the present series is not surprising. Similar observations are reported in a recent study⁴³ indicating a direct and significant correlation between arterial blood pressure and mortality rate in patients with SAH.

In paper II it is demonstrated that post-hemorrhagic CSF from patients with aneurysmal SAH may induce pronounced constriction of pial arterioles in the cat. These results support the concept that the sometimes obvious discrepancy between arterial spasm visualized by angiography and delayed cerebral ischemic dysfunction may represent regional differences in vascular response to post-hemorrhagic CSF.

In the experiments one of the CSF specimens was taken from a patient without neurological symptoms. When compared with the effects of CSF from three other patients (with delayed ischemic deficits) no obvious difference was observed. These observations may indirectly imply variations in vascular responsiveness between patients.

Paper III confirms that there are no major differences in CSF vasoconstrictor activity between patients with or without cerebral ischemic dysfunction after aneurysmal SAH. Furthermore, clinical deterioration is not paralleled by an increased vasoconstrictor activity. The generally low values for vasoconstrictor activity in the present series of early operated patients as compared to previous studies on conventionally treated cases, indicates that the operative procedure (See Methods, paper I) reduces CSF vasoconstrictor activity. The fact, that in spite of this reduction, some patients develop delayed ischemic dysfunction, provides further evidence for the existence of individual variations in cerebrovascular responsiveness to post-hemorrhagic CSF in humans.

The identity of the vasoconstrictor substance/s in CSF could not be established in the present study. However, 5-hydroxytryptamine, histamine, noradrenaline, adrenaline, acetylcholine and angiotensin_{II} were eliminated as prime vasoconstrictor agents inducing cerebral vasospasm. Potassium was also eliminated as a major vasoconstrictor factor, although there was a highly significant correlation between K^+ concentrations in CSF samples and vasoconstrictor activity. This might be explained by a concomittant release of K^+ and vasoconstrictor materials.

The results in paper IV indicate that there are marked individual variations in the response of human cerebral arteries to different vasoactive agents, plasma as well as to CSF from patients with aneurysmal SAH. In contrast, there was very little variation in the responsiveness to prostaglandin F_{2a} . The present study does not permit any conclusions about possible segmental differences in the responsiveness along one and the same cerebral artery. However, there were no obvious differences in responsiveness between vessels obtained from different cerebral territories. Factors such as age, sex and additional medication may have contributed to the variable results but none of these parameters was consistently correlated to deviation in vascular response. Thus, it seems justified to conclude that human cerebral vessels in vitro exhibit a marked individual profile in terms of reactivity towards vasoactive substances. The observations may account for the fact that delayed arterial/arteriolar narrowing does not uniformly occur after aneurysmal SAH.

Previous studies have demonstrated that an intact prostaglandin synthesis may be necessary for the maintenance of normal cerebrovascular tone, but also that disturbances of the prostaglandin synthesis are involved in

the pathogenesis of delayed cerebral vasospasm.⁴⁴ Based on the knowledge that on the one hand prostacyclin (PGI_2), a main arachidonic acid metabolite with vasodilating properties, is mainly formed by the endothelial layer, and that on the other hand experimental SAH results in progressive ultrastructural endothelial lesions, it has been proposed that vasoconstriction after SAH may be due to impaired PGI_2 synthesis. Based on this assumption, it is suggested that inhibition of prostaglandin synthesis may affect CSF-induced contractions. As shown in paper V, contractions of human pial arteries induced by CSF from SAH patients were markedly increased by interfering with the prostaglandin synthesis by use of indomethacin. In some experiments more than a ten-fold augmentation of the contractions was observed. The results suggest that the development of delayed cerebral vasospasm might be due to a local disturbance of the synthesis of PGI_2 and/or other vasodilatory arachidonic acid metabolites.

This concept is further supported by the observations that PGI_2 had a very marked relaxant effect on contractions induced by post-hemorrhagic CSF. Similar effects were observed on contractions induced by noradrenaline and 5-hydroxytryptamine while potassium-induced contractions were only partly counteracted. The mechanisms behind the relaxant effects of PGI_2 are undefined, though PGI_2 does not seem to affect a final step in the cerebrovascular smooth muscle contraction process.

The indomethacin-induced augmentation of contractions evoked by post-hemorrhagic CSF, might thus be due to inhibition of the synthesis of 'protective' arachidonic acid metabolites. One may speculate whether the observations of marked individual variations in vascular responsiveness to post-hemorrhagic CSF represent individual differences in arachidonic acid metabolism within the vessel wall.

Since the mechanisms responsible for cerebral vasospasm are unknown, it is not surprising that no specific blocking agents have emerged. Efforts have been made to counteract vasoconstriction post-SAH by interfering with the final step in the contraction process within the vascular smooth muscle cell. Despite considerable knowledge about the role of calcium in smooth muscle, there is relatively little information on the subject in human cerebrovascular smooth muscle. From experiments on myocardial muscle it has been concluded that calcium antagonistic drugs selectively inhibit calcium influx, and that their main site of action is the 'slow calcium channels' in the cell membrane.⁴⁵ On the other hand, evidence has been

presented suggesting that nifedipine also can affect later steps in the excitation-contraction processes, including inhibition of the release of intracellularly stored calcium.⁴⁶ Two types of calcium dependent activation mechanisms in smooth muscle have been suggested. One of these is correlated to spike discharges and responsible for phasic activation (P-mechanism).⁴⁷ This type of activation has been called "electro-mechanical coupling" (Somlyo and Somlyo, 1968).⁵⁰ The other activation mechanism is correlated to spike-free depolarization and responsible for tonic activation (T-mechanism),⁴⁷ corresponding to "pharmoco-mechanical coupling" (Somlyo and Somlyo, 1968).⁵⁰ It has been suggested (Bolton, 1979)⁵¹ that in the electro-mechanical coupling (P-mechanism) calcium influx occurs through membrane-potential sensitive calcium channels. These calcium channels can be effectively blocked by calcium antagonists. On the other hand, pharmoco-mechanical coupling (T-mechanism) may involve another type of calcium channel, the receptor operated calcium channel (Bolton, 1979).⁵¹ These channels can not be effectively blocked by calcium antagonists.(Fig.2)

If the statement is valid that calcium antagonists selectively block the P-mechanism and since the receptor operated channels are correlated to the T-mechanism, one would expect a less relaxant effect of nifedipine on contractions induced by amines than by potassium. This was also the case in the in vitro study presented in paper VI. Human pial arteries, contracted by potassium, were almost completely relaxed by nifedipine as well as by nimodipine while at the same concentration the calcium antagonists only reduced contractions induced by noradrenaline by 70 per cent and those by 5-hydroxytryptamine by 60 per cent. The relaxant effect on prostaglandin F_{2a} -contracted vessel segments was even less pronounced. These observations would indicate that these agents contract the vessel by stimulating calcium influx through receptor operated channels or by mobilizing intracellularly stored calcium. When compared to the observations in paper V, where prostacyclin was found to only partly counteract potassium-induced contractions, one may speculate whether prostacyclin, in contrast to nifedipine, interferes with the receptor operated channels in the vascular smooth muscle cell.

In the present study there was evidence for greater susceptibility to nifedipine of human cerebral arteries, compared to human mesenteric arteries contracted by potassium. In a previous study on dog cerebral and mesenteric arteries similar results were obtained on prostaglandin F_{2a} -induced contractions, while no differences were observed on potassium-induced

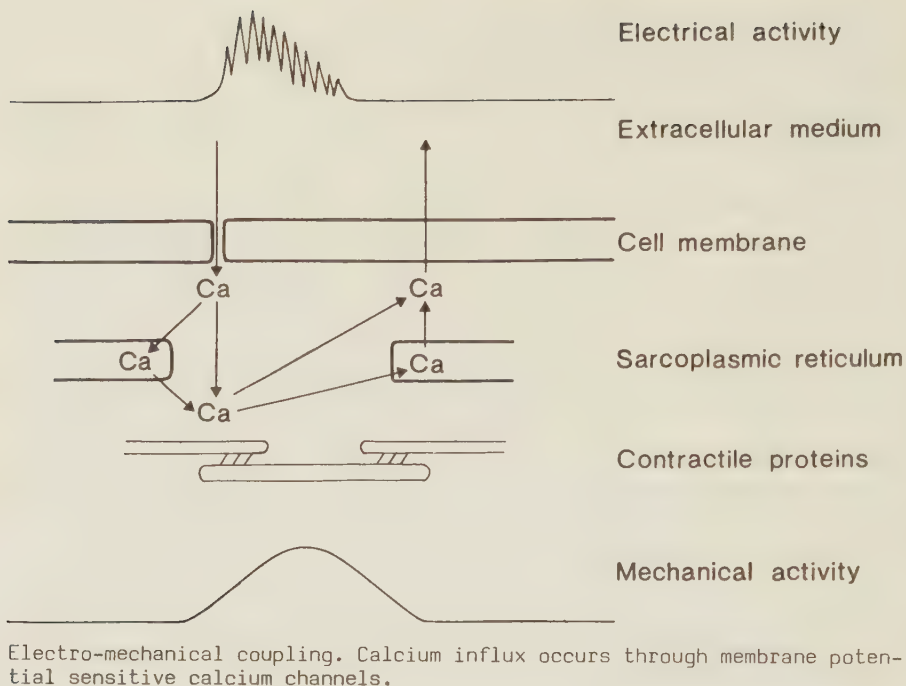
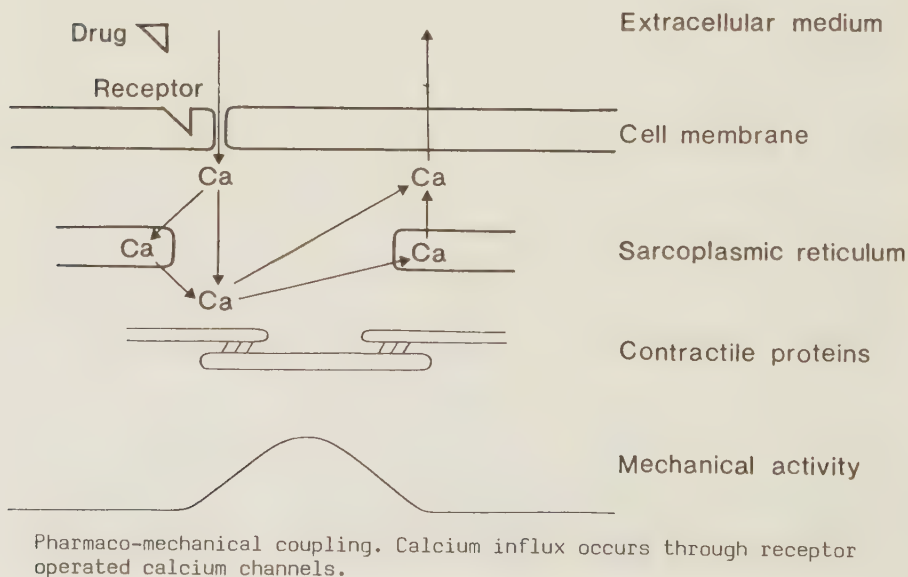


Fig. 2. (Andersson, 1981)⁵²



contractions.⁴⁸ The divergent results may be due to species differences.

The lower threshold for potassium-induced contraction of human pial arteries as compared to arteries from other vascular territories or species may be explained by differences in $\text{Na}^+ - \text{K}^+$ ATPase activity. On the other hand, Casteels⁴⁹ has suggested that changes of the membrane potential only plays a secondary role in the modulation of arterial wall tension under physiological conditions, and that potassium in low concentrations exerts its effect by affecting the vascular autonomic nerves. This, in turn, opens the possibility that the lower threshold in pial than in mesenteric arteries for potassium-induced contraction found in the present study might represent differences in the distribution of autonomic nerves between the vessels. However, the results obtained in the presence of cocaine, propranolol or prazosin do not suggest that such differences, if they exist, are of main importance.

The results in paper VII demonstrate that nifedipine, when locally applied in the subarachnoid space in vivo, is able to induce very marked concentration-dependent arteriolar dilatations. The similar though less pronounced effect on the venular side confirms the existence of contractile properties of the venular wall. About 80 per cent of the total intracranial blood volume is distributed in the venous part of the cerebral vasculature. This fact must be taken into account when calcium blockers of the nifedipine type are considered as treatment alternatives in cerebrovascular disorders.

So far we have no explanation to the biphasic sequence of arteriolar caliber changes in focal ischemia. The nifedipine-induced relaxation of constricted 'ischemic' arterioles indicates that the mechanisms behind the delayed ischemic arteriolar constriction is calcium-dependent. The occurrence of pronounced cortical vasoconstriction in focal ischemia may be of relevance not only in the pathogenesis of stroke, but also in cerebral vasospasm after SAH. Provided that proximal vasospasm in the conducting arteries is sufficient to produce distal ischemia, and that the observed phenomenon is not only a phenomenon in the cat, one might speculate whether a similar 'ischemic' vasoconstriction in cortical areas may contribute to delayed ischemic dysfunction after SAH. If the observed delayed cortical vasoconstriction is due to an increased concentration of potassium in the perivascular space then the lower threshold observed for potassium-induced contractions on human cerebral arteries (See Paper VI) might lead to an aggravation of this phenomenon in humans.

CONCLUSIONS

Early aneurysm surgery (within approximately 60 hours post-SAH) is superior to conventional delayed treatment. Such management prevents repeat subarachnoid hemorrhage but does not dramatically reduce the incidence of delayed cerebral ischemic dysfunction. Pre-existing arterial hypertension is a negative prognostic factor.

In vivo, post-hemorrhagic CSF induces pronounced constriction of arterioles, i.e. resistance vessels not discernible on arteriograms. These constrictions are effectively blocked by nifedipine.

There is no close relationship between vasoconstrictor activity in post-hemorrhagic CSF and clinical condition or angiographic vasospasm, i.e. delayed deterioration or development of angiographic vasospasm is not paralleled by increased CSF vasoconstrictor activity. Removal of subarachnoid blood clots and blood-contaminated CSF in early surgery results in generally low CSF vasoconstrictor activity.

In vitro, human pial arteries show marked individual variations in the response to post-hemorrhagic CSF, plasma, and amines. These findings may be relevant for the capricious appearance of cerebral vasospasm.

In vitro, contractions of human pial arteries induced by CSF from SAH patients are markedly increased by interfering with the arachidonic acid metabolism by use of indomethacin. The results suggest that the development of delayed cerebral vasospasm may be due to a local disturbance of the synthesis of vasodilatory arachidonic acid metabolites, further supported by the observations that prostacyclin has a pronounced relaxant effect on contractions induced by post-hemorrhagic CSF.

Potassium, amines and prostaglandin F_{2a} activate isolated human pial and mesenteric arteries by different calcium-dependent mechanisms. The calcium antagonists nifedipine and nimodipine have pronounced relaxant effects on human pial arteries contracted by potassium, amines and PGF_{2a} , being most potent on potassium-induced contractions. The threshold for potassium-induced contraction is 3 to 5 mM lower in human pial as compared to human mesenteric arteries.

In vivo, topical application of nifedipine causes marked concentration-dependent pial arteriolar dilatation, increasing with decreasing vessel size. Nifedipine induces pial venular dilatations as well, which however are less pronounced but more longlasting. Nifedipine also dilates arterioles in ischemic cortex, constricted following MCA occlusion.

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Results of early operations for ruptured aneurysms

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✓ In a consecutive series of 219 patients with a ruptured aneurysm of the anterior part of the circle of Willis, 119 patients (54%) made a good recovery and 67 (31%) died. Of 53 patients who did not have surgery, six (11%) made a good recovery and 37 (70%) died. Urgent surgery with evacuation of an associated significant intracerebral hematoma was performed in 30 patients; nine (30%) made a good recovery and 15 (50%) died. Delayed surgery was performed in 55 patients of whom 42 (76%) made a good recovery and two (4%) died. Early intracranial operation (within 48 to 60 hours after subarachnoid hemorrhage (SAH)) was performed in 81 patients who were in Grades I to III prior to surgery. Sixty patients (74%) made a good recovery, and eight died within a month. Five patients were severely disabled and died 2 to 8 months after SAH and surgery. In 17 patients, although the immediate postoperative course was uneventful, evidence of cerebral ischemia developed 4 to 13 days after the bleed and resulted in death in eight patients. A poor outcome was correlated with a history of elevated blood pressure before SAH. Seven patients, of whom six were women of child-bearing age, demonstrated pronounced vasospasm on postoperative angiography; nevertheless, they remained well and free from ischemic symptoms after surgery.

Early operation combined with removal of subarachnoid clots and rinsing the basal cisterns does not eliminate the risk of delayed ischemic dysfunction. Such early surgery, however, improves overall outcome by preventing recurrent bleeding, and may also reduce the frequency of hydrocephalus.

KEY WORDS • cerebral vasospasm • ischemic deficit • early aneurysm surgery • perivascular blood collection • arterial hypertension

THE choice of timing of surgical intervention for ruptured intracranial aneurysms has caused controversy for many years. In 1953, Norlén and Olivecrona²⁹ concluded that intracranial operations for ruptured aneurysms were extremely dangerous during the acute phase, and advised that surgery should be delayed until after the 3rd week of the acute illness. On the other hand, Pool³¹ pointed out in 1961 that the mortality rate need not be forbiddingly high in young patients in good condition.

The first observation that angiographic spasm does not develop immediately after subarachnoid hemorrhage (SAH) but only at some time toward the end of the 1st week was made by Kägström, *et al.*,²¹ who then advocated that surgery should be performed within 48 hours of rupture, even if the mortality rate approached 20%.²⁰ Symon⁴⁴ similarly concluded that the "operation should be undertaken as soon as the clinical condition of the patient permits . . ."

The value of early (within approximately 48 hours after rupture) as opposed to late (10 days or more after rupture) operations for intracranial aneurysms

remains controversial. It appears that most surgeons^{3,4,10,11,14,15,26,29,39,40} still favor a delayed operation as the best method of reducing postoperative ischemic complications. However, early surgery is proposed increasingly often,^{16-18,23,24,34-36,41,46,47,49,50} in an attempt to improve overall outcome not only by preventing a rebleed but also by avoiding ischemic dysfunction by removal of subarachnoid blood.

On the basis of these views, since August, 1976, we have operated within 48 hours after the SAH on patients in preoperative neurological Grades I-III of Hunt and Hess.¹⁷ In a few cases, surgery was postponed until the 3rd day after SAH. Postoperative angiography was performed in all cases, with the exception of three patients who died in the early postoperative period. We have reviewed our experience to discover the results of this form of management.

Clinical Material

This report focuses on the results in a consecutive series of 81 patients, who presented between August,

TABLE 1

Site of ruptured aneurysm and results in total series
of aneurysm patients with early surgery*

Outcome	ACoA	ICA	MCA	Total Cases	
				No.	Percent
good recovery	22	25	13	60	74
fair result	4	3	1	8	10
dead	8	3	2	13	16
total cases	34	31	16	81	100

*ACoA = anterior communicating artery; ICA = internal carotid artery; MCA = middle cerebral artery.

1976, and June, 1980, with a ruptured saccular aneurysm of the anterior part of the circle of Willis, and who were operated on within 48 to 60 hours after SAH. None of these patients had marked associated intracerebral hematoma.

During those years, we did not perform surgery in the interval 2½ to 8 days after aneurysm rupture. Delayed surgery for ruptured aneurysms of the anterior part of the circle of Willis (more than 8 days after SAH) was performed in 55 patients, of whom 32 had been admitted too late for early surgery and 23 had not been selected for such surgery. Thirty patients presented with an intracerebral hematoma requiring urgent evacuation. For various reasons, 53 patients were not subjected to aneurysm surgery.

The operation was performed via the pterional approach described by Yaşargil and Fox.⁴⁹ The arachnoid of the carotid and chiasmatic cisterns and, in many cases, the proximal Sylvian fissure was opened by sharp dissection. Any blood clots were removed and the anterior cisterns were irrigated with normal saline in order to remove blood-contaminated cerebrospinal fluid (CSF). The interpeduncular and pontine cisterns were also rinsed with saline via a catheter that was introduced through a perforation of Liliequist's membrane.²⁸

Results were determined at follow-up examination 6 months to 4 years after surgery. Patients who had no neurological deficits and had returned to full previous activity were considered to have a "good recovery."

TABLE 2

Age and sex distribution in patients with early surgery,
with operative outcome

Age (yrs)	Good Recovery		Fair Result		Dead	
	M	F	M	F	M	F
10-19	2	0	—	—	—	—
20-29	3	7	—	—	1	—
30-39	7	6	—	1	—	—
40-49	7	6	—	1	2	2
50-59	6	7	1	2	3	1
60-69	3	5	1	2	—	2
70-79	1	0	—	—	1	1
total	29	31	2	6	7	6

Patients who were at home and capable of self care, but who had persisting neurological deficit or mental disturbances, were classed as having a "fair result." Severely disabled patients, totally dependent on others, were classed as having a "poor result." Patients who were severely disabled or survived in a more or less vegetative state and who later died were classed among the deaths even if the immediate cause of death was not ascribable to SAH, but for example to pulmonary embolism.

Operative Results

Early Surgery

The site of aneurysm and the results in the total series of patients operated on early are shown in Table 1.

Good Recoveries. The result was regarded as good in 60 patients (74%). Age and sex distribution in this group is given in Table 2. Two patients were classed as having a good recovery although they remained with neurological deficit (diplopia and oculomotor nerve palsy, respectively) due to preoperative damage from aneurysm rupture.

Of five patients in this group who had arterial hypertension prior to SAH, four were confused for a considerable time after surgery. Among the remaining 55 cases, two women (aged 30 and 32 years) with middle cerebral artery (MCA) aneurysms had angiographic evidence of severe vasospasm and delayed ischemic deficits. Both patients recovered from their ischemic deficits. Seven patients demonstrated severe vasospasm at angiography performed between the 7th and 10th day after SAH, but they remained symptom-free. Six of these patients were women of child-bearing age.

Out of 21 patients in whom the ventricular fluid pressure (VFP) was monitored according to Lundberg's technique,²⁷ there was a need for CSF drainage in 12 cases. Drainage varied from intermittent for 1 to 2 days to continuous for 14 days, according to the regimen described by Sundbärg and Pontén.³⁰ Three of these patients had preoperative CSF drainage, which led to obvious improvement and made early surgery appropriate according to the above criteria. Absorption of CSF did not become permanently impaired in any patient in this group.

Fair Results. The operative result was defined as fair in eight patients with early surgery. The age and sex distribution is given in Table 2. Neurological dysfunction included delayed ischemic deficits in six cases. Two patients developed impaired CSF absorption and had a ventriculoatrial shunt implanted 2 and 2½ months after SAH, respectively. Intracranial pressure (ICP) had been monitored during the postoperative period in one of these patients, demonstrating a slight elevation of VFP. Two patients needed postoperative ventricular CSF drainage, and

Early aneurysm surgery

TABLE 3

Site of ruptured aneurysm and results in 17 patients with early surgery and delayed ischemic deficits*

Outcome	ACoA	ICA	MCA	Total Cases
good		1	2	3
fair	3	3		6
dead	4	2	2	8
total	7	6	4	17

*ACoA = anterior communicating artery; ICA = internal carotid artery; MCA = middle cerebral artery.

one of them was later reexamined because of suspected hydrocephalus, which was not verified.

Deaths. There were 13 deaths in the series of patients with early surgery. Eight were early (within 1 month after SAH) and five late (2 to 8 months after SAH). Table 2 shows the age and sex distribution.

Eight patients, two with internal carotid artery (ICA) aneurysms, two with MCA aneurysms, and four with anterior communicating artery (ACoA) aneurysms, were in the same grade immediately after surgery as just before. However, after an interval of 2 to 7 days, that is, 4 to 8 days after SAH, they deteriorated. Six of these patients had arterial hypertension prior to SAH, and all except one received antihypertensive medication at the time of SAH.

In four cases, the cause of death was more or less occasioned by a technical failure. A 57-year-old hypertensive man with an ACoA aneurysm was found at autopsy to have a clip positioned so that circulation in the left anterior cerebral artery was obstructed. A 72-year-old hypertensive woman with a right ICA aneurysm was found on postoperative angiography performed 3 days after SAH to have occlusion of the choroidal artery. A 66-year-old woman with an ACoA aneurysm had a broad "spontaneous" rupture of the ICA while the position of a brain retractor was adjusted. The fourth patient, a 55-year-old man with an upward-directed broad-based ACoA aneurysm, did not tolerate trapping of the aneurysm.

Neurological deficits persisted in one patient, who was found at autopsy to have a focal infarction. Among the patients in this group, eight had ventricular CSF drainage. Another three patients had ICP monitoring, but no need for drainage was identified.

Delayed Ischemic Deficits and Outcome. In the present series, 17 (21%) of the 81 patients with early surgery developed delayed ischemic deficits with onset of symptoms 4 to 13 days after SAH. The site of ruptured aneurysm and results at follow-up examination are given in Table 3. Eight of these patients died (see above); however, two of these had more than one aneurysm and had surgery directed toward an unruptured aneurysm. Preoperative computerized tomography (CT) and angiography had failed to identify

TABLE 4

Site of aneurysm and results in 18 patients with early surgery who had arterial hypertension prior to SAH*

Outcome	ACoA	ICA	MCA	Total Cases
good	3	2	1	6
fair	1	1	1	3
dead	5	3	1	9
total	9	6	3	18

*SAH = subarachnoid hemorrhage; ACoA = anterior communicating artery; ICA = internal carotid artery; MCA = middle cerebral artery.

which aneurysm had ruptured. Consequently, subarachnoid washout in the vicinity of the ruptured aneurysm was not performed in these two cases. A third patient, who died with delayed ischemic deficits, had had an SAH and aneurysm surgery 12 years earlier. In this case, there were pronounced subarachnoid adhesions, and it was not even possible to clean the anterior cisterns.

In six cases, the outcome was defined as fair and in three as good at follow-up review.

Arterial Hypertension and Outcome. Eighteen patients with early surgery were categorized as hypertensive (diastolic pressure exceeding 110 mm Hg on at least two separate measurements before SAH, or a history of previous treatment for arterial hypertension). The site of ruptured aneurysm and result at follow-up for these patients are presented in Table 4. Among the 18 hypertensive patients, nine (50%) developed delayed ischemic deficits in contrast to eight of the 63 normotensive patients (13%). Of the eight hypertensive patients who received antihypertensive medication at the time of SAH, six (75%) developed delayed ischemic deficits.

Altogether, nine (50%) of the hypertensive patients died. Two deaths were due to operative failures, in one case surgery was directed toward an unruptured aneurysm, and in a fourth a focal infarction was found at autopsy (see above). Six patients woke up in the same grade as before surgery but deteriorated between the 4th and 8th day after aneurysm rupture. Among the eight patients with a fair outcome, three (38%) were hypertensive. Of the six hypertensive patients finally defined as having a good recovery, only two had not manifested transient neurological deficits. The final outcome for hypertensive versus normotensive patients is summarized in Table 5.

Daily Lumbar Puncture. Of the early surgery group, 42 patients had daily lumbar punctures postoperatively for an average of 5 days in order to aid in removal of blood-contaminated CSF. The volumes evacuated daily ranged from 20 to 30 ml. Lumbar punctures resulted in a lowered VFP for a few hours. The withdrawal of lumbar CSF had no obvious effect on the incidence of delayed ischemic deficits.

TABLE 5
Outcome in patients with early surgery, with
arterial hypertension versus normotension*

Outcome	Hypertensive		Normotensive	
	No.	Percent	No.	Percent
good & fair	9	50	59	94
dead	9	50	4	6
total	18	100	63	100

*The difference in outcome between normotensive and hypertensive patients is significant at $p < 0.001$ (Fisher's exact test).

Urgent Surgery

Of the 30 patients who required emergency surgery due to the presence of an associated significant intracerebral hematoma, nine made a good recovery, three were classed as fair, and another three as poor results at follow-up review. Fifteen of these patients died (Table 6).

Delayed Surgery

The results in the delayed surgery group are summarized in Table 6. Of the 32 patients who were admitted too late for early surgery but who were subjected to late surgery, 26 made a good recovery, two were classed as fair, and three as poor results; one patient died. Of the 23 patients who were not selected for early surgery, no aneurysm was visualized on the initial angiogram in two patients, both of whom made a good recovery after delayed surgery. Of the remaining 21 patients who were not selected for early surgery but later improved and had delayed surgery, 14 made a good recovery, four were classed as fair, and two as poor results, while one patient died.

In summary, 76% of the patients with delayed surgery made a good recovery, 11% were classed as fair and 9% as poor, and 4% died.

No Surgery

The outcome for the 53 patients who did not have aneurysm surgery is shown in Table 6: 23 patients would have fulfilled the criteria for early surgery; six patients were considered "borderline patients" (that is, patients arriving in time for early surgery but not operated on because of various complicating factors, like being over 70 years old, in combination with advanced pulmonary emphysema, heart dysfunction, or severe arteriosclerotic narrowing of main cerebral arteries); and 24 patients remained in Hunt and Hess' Grades IV or V or deteriorated during the first 12 to 24 hours after SAH, and were at no time considered candidates for aneurysm surgery.

The 23 patients who would have fulfilled the criteria for early surgery but were not operated on were further analyzed. As shown in Table 7, nine patients did not have early surgery as a consequence of too late admission due to patient's delay; poor results were caused by the development of delayed ischemic dysfunction in one case and in combination with repeat SAH in one case; one death was caused by delayed ischemic dysfunction, and two deaths were caused by repeat bleeds. Doctor's delay (that is, delay at a local hospital) made early surgery impossible in eight cases: two patients died of delayed ischemic deficits, and two of rebleeds; one poor result was caused by delayed ischemic dysfunction. Neurosurgical delay (that is, organizational problems, patient's disease primarily misinterpreted) prevented early surgical intervention in six patients, of whom four died, all of rebleeds within 10 days after the first SAH. One

TABLE 6
Overall outcome in 219 patients with a ruptured aneurysm of the anterior part of the circle of Willis

Patient Groups*	Good Recovery	Fair Result	Poor Result	Deaths	Total Cases
urgent surgery	9	3	3	15	30
early surgery	60	8	—	13	81
delayed surgery					
not selected for early surgery	16	4	2	1	23
fulfilled criteria for early surgery but admitted too late	26	2	3	1	32
no surgery					
fulfilled criteria for early surgery during first 60 hrs post-SAH	5	1	4	13	23
borderline status	1†	—	—	5	6
Hunt & Hess' Grades IV and V	—	1	4	19	24
total cases	119	18	15	67	219
percent	54	8	7	31‡	100

*For fuller description see text.

†Follow-up period: 2½ years.

‡Management mortality.

Early aneurysm surgery

poor result was a consequence of persisting delayed ischemic dysfunction.

Of the 23 patients who fulfilled the criteria for early surgery but were not operated on, 13 patients died. Five of these 13 patients (38%) were hypertensive.

Discussion

Our policy is to have all patients with a verified or suspected SAH referred immediately irrespective of neurological grade. This policy is probably reflected in our management mortality, which was 31% for the total series of 219 cases with a ruptured aneurysm of the anterior part of the circle of Willis.

The outcome for the group of patients receiving early surgery (81 cases) versus the outcome for the group of patients who fulfilled the criteria for such surgery but who were not operated early (55 cases) is summarized in Table 8. Our results indicate that early surgery is equal or superior to delayed treatment. In fact, when considering good recoveries only, the results of early surgery were fully comparable to the results in the selected group of patients subjected to conventional delayed surgery (74% and 76% good recoveries, respectively). Early surgery is also accompanied by a markedly shortened hospital stay: in our series, the 60 patients with good recoveries in the group operated on early spent an average of 16 days in the clinic in contrast to more than double that time for patients receiving delayed therapy.

When compared to the probability data of Alvord, *et al.*,² for a 2-year survival period with conservative treatment, the results in the present series with early operation indicate that early aneurysm surgery is superior to the natural history of the disease. Only 55% to 65% of the patients in our series with early operation would be alive after 2 years.

We discovered a striking correlation between the presence of arterial hypertension prior to SAH and development of delayed ischemic deficits. In 1963, Allcock and Drake¹ reported that systemic hyperten-

sion was accompanied by an increased incidence of angiographic vasospasm, and concluded that arterial spasm worsened the prognosis. Wilkins, *et al.*,⁴⁸ found a slightly increased frequency of angiographic evidence of vasospasm in hypertensive compared to normotensive patients. The 1966 Cooperative Study¹⁴ revealed that the category of patients with preexisting arterial hypertension had a 55.3% mortality, as compared to the overall mortality of 36.8% in the whole series. Fisher, *et al.*,¹⁰ found no correlation between vasospasm and arterial hypertension in their analysis, and Samson, *et al.*,³⁸ in their series of 106 consecutive Grades I and II patients, found no correlation between preexisting arterial hypertension and the development of postoperative neurological deficit.

Several studies indicate that arterial vessels in hypertensive individuals differ morphologically from those of normotensive individuals.^{5,12,28} Cook and Yates⁶ found medial hypertrophy at all artery sizes in brains from hypertensive humans. Russell³² examined brains from patients who had hypertension during life and found that small penetrating arteries, 50 μ in diameter, showed decreased lumen and increased wall thickness. Spontaneously hypertensive experimental animals are more vulnerable than normotensive animals to bilateral ligation of the common carotid artery, which may indicate a reduced capacity for compensatory vasodilatation.¹³ Furthermore, the cerebrovascular resistance during maximum vasodilatation is considerably higher in hypertensive rats than in normotensive rats.¹⁹ This suggests that hypertensive patients may be more vulnerable than normotensive patients to severe arterial narrowing, especially when this narrowing (spasm) affects the penetrating arteries.

In a recent report,³³ it was stated that patients with angiographically demonstrated vasospasm eventually show neurological symptoms. The findings in our series are not in agreement with this statement. Some patients in the present series developed severe angiographic vasospasm (Type 1³³) but remained free from symptoms and signs of delayed ischemic dysfunction. Other patients with severe delayed ischemic deficits demonstrated only a very moderate narrowing of the larger cerebral arteries at angiography. These find-

TABLE 7

Outcome in 23 patients who fulfilled criteria for early surgery but were not operated on

Reason for Delay	Good Recovery	Fair Result	Poor Result	Deaths	Total Cases
patient's delay	1*	1	2	5	9
doctor's delay	3†	—	1	4	8
neurosurgical delay	1‡	—	1	4	6
total	5	1	4	13	23

*Four-year follow-up period.

†One patient died 1½ years later of repeat subarachnoid hemorrhage (SAH) (left internal carotid artery aneurysm); one patient had a 2-year and 2-month follow-up period; and one patient had no filling of the aneurysm on repeat angiogram 2½ and 1 year post-SAH (anterior communicating artery aneurysm).

‡Six-month follow-up period.

TABLE 8

Outcome for patients with early surgery versus outcome for patients not operated on early

Outcome	Early Surgery Patients	Patients Not Operated on Early*
good recovery (%)	74	56
morbidity (%)	10	18
mortality (%)	16	26
total	81	55

*Patients who would have fulfilled criteria for early surgery but were not operated on early.

ings stress the importance of the distribution of vasospasm and the adequacy of the patient's compensatory mechanisms, and demonstrate that spasm in the conducting arteries is only one factor in the total equation that determines how well the brain is perfused after SAH.⁴⁷

Disturbance of autoregulation^{40,42} and intravascular platelet aggregation with embolization of platelet thrombi⁴³ and consequent occlusion of arterioles and capillaries may occur as a consequence of extravasation of blood into the perivascular space.^{8,22,37,45} By assessing the amount of subarachnoid blood visualized on CT, Fisher, *et al.*,⁹ found that blood localized in the subarachnoid space is the only etiological factor in vasospasm. Consequently, it has been proposed that early and extensive removal of perivascular blood collections should be effective in minimizing the occurrence of vasospasm and delayed ischemic deficits.^{34,36,41,46,47} Our results fail to provide evidence that the capricious appearance of cerebral delayed ischemic dysfunction can be eliminated by our present operative procedures. However, this does not preclude the fact that subarachnoid blood is the prime etiological factor in vasospasm and delayed cerebral ischemia. In cases in which a preoperative CT scan showed a thick layer of subarachnoid blood and in which an early postoperative CT scan was performed, there was consistently residual blood. With improved techniques for cleaning the subarachnoid spaces, it might be possible to further reduce the incidence of delayed ischemic complications.

In a recent series of aneurysms operated on early,⁴¹ a high percentage of patients required a ventriculoperitoneal shunt because of impaired CSF outflow. In our series, temporary CSF drainage by catheter to a frontal horn was performed in 22 cases. In many instances, CSF drainage was started with the onset of delayed ischemic deficits. Only two patients (3%) developed permanently impaired CSF outflow according to clinical criteria, verified by CT scan and lumbar infusion test. Both patients had a ventriculoatrial shunt implanted, and at follow-up review they were considered as having a fair outcome. Samson, *et al.*,³⁶ in their series of 106 good-risk surgical patients (79 operated on within 8 days after SAH and 27 operated on later), reported a 9% incidence of postoperative hydrocephalus. In our opinion, early aneurysm surgery and washout of blood-contaminated CSF lessens the risk of subarachnoid fibrosis and impaired CSF outflow.

The case for delayed surgery was strongly stated by Drake:⁶ "if we could learn how to keep a patient safe from rebleeding for a week . . . the problems of the surgery of ruptured intracranial aneurysms would be nearly solved." In arriving at this view, he was probably influenced both by the extremely poor results of early surgery and also by his observations of the condition that his associates had faced when performing early aneurysm surgery: "it was not all spasm either,

for I watched them struggling with the angry, swollen brain."¹⁷

Today, with microsurgical adjuncts and improved techniques, dealing with the blood-stained and swollen brain certainly is not easy, yet does not imply a disastrous struggle. Future improvements in the outcome of patients with SAH may depend upon a rephrasing of Drake's statement: if we could learn how to keep a patient safe from developing delayed cerebral ischemic dysfunction, then the problem of surgery of ruptured aneurysms would be nearly solved.

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Vasoconstrictive effects of human post-hemorrhagic cerebrospinal fluid on cat pial arterioles *in situ*

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✓ Cat cortical arterioles were exposed *in vivo* to cerebrospinal fluid (CSF) from four patients with subarachnoid hemorrhage (SAH) due to a ruptured intracranial aneurysm. Pial arteriolar caliber was measured by the television image-splitting technique. There was a consistent vasoconstrictive response to CSF. This effect could be ascribed neither to the pH of the CSF nor to the potassium concentration. The vasoconstriction, which was more pronounced with decreasing arteriolar caliber, could be resolved by the perivascular application of nifedipine.

KEY WORDS • subarachnoid hemorrhage • post-hemorrhagic cerebrospinal fluid • vasoconstriction • nifedipine

RUPTURE of an intracranial aneurysm is frequently complicated by delayed arterial spasm and cerebral ischemia.^{1,11,12,14,15,17,19,21,23,24,26,27} The relationship between these events is unclear because it is recognized that delayed cerebral ischemia after subarachnoid hemorrhage (SAH) does not always have an angiographic correlate, and that arterial spasm visualized by angiography does not necessarily mean ischemic dysfunction. These observations indicate that the state of the resistance vessels is of prime importance in the pathogenesis of delayed cerebral ischemia after SAH.

In vitro studies^{2,6,7,9,18,28} have shown that cerebrospinal fluid (CSF) from patients with SAH may induce constriction of isolated major cerebral arteries. Information on the *in vivo* effect is sparse since there is only one report²² on the effects of CSF from patients with SAH on small cerebral arteries and arterioles *in situ*.

The present study had two purposes. The first was to discover the effect of post-hemorrhagic human CSF on minor cerebral arteries and resistance vessels *in vivo*. The second arose from our previous observation that perivascular microinjections of the calcium-antagonistic drug nifedipine (Adalat) markedly dilates both normal pial arterioles and vessels exposed to perivascular blood;⁸ we therefore set out to investigate the effect of nifedipine on any vasoconstriction that might be induced by a patient's CSF.

Material and Methods

Clinical Material

Samples of CSF were obtained from four patients suffering from SAH due to a ruptured aneurysm. Relevant clinical details are summarized briefly in Table 1. Three of the patients (A, B, and C) developed severe delayed neurological deficits, considered to be ischemic in origin. In these patients, ventricular fluid pressure (VFP) was continuously monitored and ventricular CSF was drained at a VFP exceeding 15 to 20 mm Hg. In no case did the arterial pressure at any time drop below 120/70 mm Hg. The fourth patient (D) remained well and without neurological symptoms, even though angiography 9 days post-SAH and 8 days after successful clipping of a right internal carotid artery (ICA) aneurysm revealed pronounced spasm in all large intracranial arteries within the territory of the right ICA.

Methods

The CSF samples were divided into 1- to 2-ml portions in glass tubes and frozen at -20°C immediately after withdrawal. The CSF was only rewarmed immediately prior to an experiment. Because the micropipettes used in these experiments (see below) are blocked by particles with a diameter exceeding 8 to 10 μ , the CSF samples were filtered through a milli-

TABLE 1
Clinical summary of four cases of ruptured intracranial aneurysms*

Feature	Patient A	Patient B	Patient C	Patient D
age (yrs), sex	48, F	44, F	51, F	37, F
aneurysm site	Bif-BA, 2 lt PCA	lt ICA	rt ICA	rt ICA
source of CSF	ventricle	ventricle	ventricle	lumbar theca
interval between last SAH & CSF sampling	15 days	13 days	9 days	7 days
interval between last SAH & onset of signs of cerebral ischemia	6 days	6 days	6 days	no signs
clinical condition at time of CSF collection	drowsy; rt hemiparesis; tachycardia; normal BP; equal size of pupils; normal pupillary light reflexes; T: 38.5°C	semicomatose; rt hemiparesis; normal PR and BP; equal size of pupils; normal pupillary light reflexes; afebrile	semicomatose; lt arm monoplegia; normal PR and BP; equal size of pupils; normal pupillary reflexes; afebrile	no neurological deficits; normal T, PR, and BP
late angiogram	ND	ND	ND	pronounced spasm on Day 9 post-SAH
drug treatment	none	alprenolol (Aptine) 200 mg × 2; hydralazine hydrochloride (Aposoline) 50 mg × 2; hydrochlorothiazide (Esidrix) 25 mg × 1	betamethasone (Betapred) 4 mg × 4 im; phenytoin (Fenantoin) 100 mg × 3	none
additional comments	first SAH 3 weeks earlier; also had lt fronto-parietal AVM	hypertensive; also had "minor leak" 3 days before major SAH	SAH 17 days earlier; this SAH occurred from intraoperative aneurysm rupture during clipping	aneurysm clipped 1 day after SAH; postoperative course uncomplicated

*Abbreviations: F = female; CSF = cerebrospinal fluid; SAH = subarachnoid hemorrhage; Bif-BA = bifurcation basilar artery; PCA = posterior cerebral artery; ICA = internal carotid artery; AVM = arteriovenous malformation; PR = pulse rate; BP = blood pressure; T = body temperature (rectal); ND = not done.

pore filter (pore size 0.22μ)* after being thawed.

The pH and the potassium concentrations of the CSF specimens were determined before the experiments (Table 2). Control human cerebrospinal fluid (CSF_E and CSF_F) was obtained from two patients who were undergoing myelography. The pH of these samples was adjusted to 7.18 according to the procedure described by Wahl, *et al.*²⁰

Mock CSF had the following composition: Na⁺ 156 mM, K⁺ 3 mM, Ca²⁺ 1.5 mM, Cl⁻ 151 mM, and HCO₃⁻ 11 mM. The final pH of the mock CSF solution was adjusted to the pH of each patient's CSF specimen, and called mock CSF_{A,B,C,D}, respectively. Mock CSF_{A,B,C,D} solutions were stored under oil and used within an hour after preparation.

The experiments were performed on 10 anesthetized cats of either sex, weighing 1.8 to 3.2 kg. Anesthesia was induced by a combination of alpha-xolone (6.75 mg/kg) and alpha-dolone acetate (2.25 mg/kg) administered intravenously. After endotracheal intubation, cannulas were inserted via the femoral vessels into the abdominal aorta and inferior

vena cava. Anesthesia was maintained with a 1% solution of alpha-chloralose (60 mg/kg) given intravenously. Additional alpha-chloralose was given as needed to abolish the corneal reflex. Oxygen was delivered via a respiratory pump with the rate and volume adjusted to maintain normocapnia. In seven animals, gallamine (3 mg/kg) was administered intravenously in order to control respiration. The femoral arterial cannula was used for continuous electro-manometric measurement of the arterial blood pressure and for removal of blood samples, which were analyzed for PaCO₂, PaO₂, pH, and base deficit. Any base deficit was corrected with 8.4% sodium bicarbonate given by slow intravenous injection. End-tidal CO₂ concentration was monitored continuously and displayed on a Satham capnograph.† Body temperature was maintained at 37° to 38°C by means of a heating blanket.

The head of the cat was placed in a stereotaxic holder, and a unilateral craniotomy, 3 × 2 cm in area, was performed over the left cerebral hemisphere with a saline-cooled dental drill. After removal of the bone

*Millipore filter manufactured by Millipore-Swinnex 13, Molsheim, France.

†Satham capnograph manufactured by Satham Instruments, 2230 Satham Boulevard, Oxnard, California.

Vasoconstrictive response to human CSF in cats

TABLE 2

Concentrations of pH and potassium in patient cerebrospinal fluid (CSF)

Sample	pH	K ⁺
CSF _A	6.4	2.6
CSF _B	7.5	2.4
CSF _C	7.9	2.1
CSF _D	8.3	2.1

flap, the dura was bathed in mineral oil before being incised and reflected laterally. The exposed cortex was continuously irrigated with heated mineral oil maintained at a temperature of 37°C.

Pial arteriolar caliber was measured by the television image-splitting technique, originally described by Baez⁵ and modified by Wahl, *et al.*,²⁶ and MacKenzie, *et al.*²⁰ The pial vessels were visualized with a Bausch and Lomb stereomicroscope at a magnification of either $\times 40$ or $\times 70$. To measure the arteriolar caliber, the image was passed through an image-splitting device situated on the vertical eyepiece of the microscope to an attached light-sensitive television camera and was displayed on a videorecorder. The shearing screw of the eyepiece, which controls the degree of image-splitting, was connected to a sensitive potentiometer and, in turn, a pen recorder. Thus, frequent measurements of shear, which is directly proportional to the vessel caliber, could be obtained. The surface of the brain was illuminated with the use of a cold, fiberoptic light source. The system was calibrated against monofilament nylon sutures of known uniform diameter before each experiment. Thus, the absolute diameter in micrometers could be calculated. It has previously been demonstrated that using this system, and under steady-state conditions, the coefficient of variation between repeated measurements of pial arteriolar diameter is 1%.²⁰

Sharpened glass micropipettes with a tip diameter of approximately 8 μm were filled with the solutions under investigation. The tops of the micropipettes were sealed with mineral oil and the tips submerged in the bulk of the solution and sealed under oil. This maneuver minimized any change in pH of the solution in the pipettes, which were used as soon as possible after preparation.

The solution was applied to a cortical arteriole by inserting the micropipette into the subarachnoid space adjacent to the vessel. The micropipettes were positioned in the perivascular space using a micro-manipulator and held in place by a pipette holder mounted on the manipulator. A hydraulic syringe was used to inject approximately 2 μl of the solution into

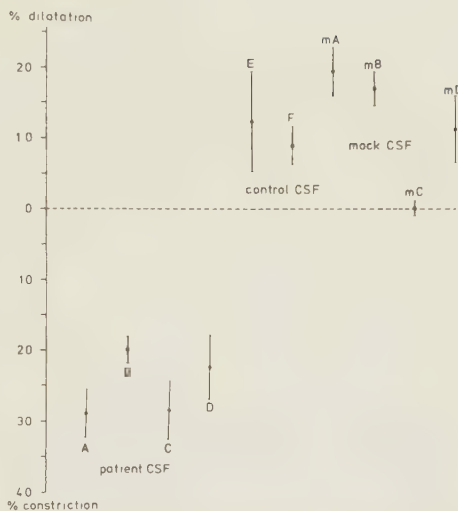


FIG. 1. Arteriolar response to patient and control cerebrospinal fluid (CSF) applications expressed as a percentage deviation from resting vessel caliber. See text. The bars indicate mean values $\pm$ SEM.

the perivascular space. Arteriolar caliber was measured continuously following the injection of the solutions, and the maximum response was compared to the preinjection diameter.

Nifedipine, in a concentration 0.1 mg/ml, for vehicle composition,⁸ was diluted in mock CSF to a concentration of 20 $\mu\text{g}/\text{ml}$. The pH of the nifedipine solution was adjusted to 7.18. To prevent decomposition of the nifedipine, the solutions were protected from light exposure except during the perivascular injections. When the CSF-induced vasoconstriction had reached a plateau, nifedipine was injected and the vascular response studied and compared to that of control vessels.

The methods of perivascular microinjection of test substances and measurement of cat cortical pial arteriolar caliber were essentially similar to the techniques described by Harper and MacKenzie.¹⁶

Results

Resting diameters of the investigated vessels were in the range of 44 to 336 μm .

All perivascular microinjections were performed at normocapnia (5.2 ± 0.4 kPa) and normotension (114.2 ± 7.1 mm Hg). Application of mock CSF_{A,B,D} invariably induced vasodilatation, whereas mock CSF_C did not cause any significant change in vessel diameter (Fig. 1). The dilatatory responses to mock CSF_{A,B,D} became more pronounced as the size of the arteriole studied decreased. Control CSF_{E,F} also caused dilatation, as shown in Fig. 1.

‡Image-splitting device manufactured by Vickers Ltd., Breakfield, Coulsden, Surrey, England.

§Schott fiberoptic apparatus manufactured by E. Leitz (Instruments), Ltd., 48 Park Vale Street, Luton, England.

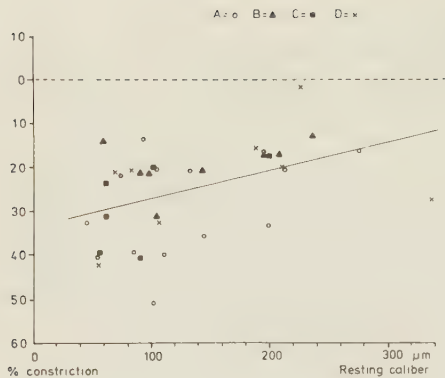


FIG. 2. The individual arteriolar responses to the four patient cerebrospinal fluid samples expressed as a percentage deviation from resting vessel caliber. Note the more marked constriction with decreasing vessel caliber. The slope of the regression line is significantly different from zero ($p < 0.01$). Equation for the regression line: $Y = A + B \cdot X$.

The vascular effects of the CSF from the four SAH patients were studied on 36 individual cat pial arterioles (CSF_{A,B,C,D} on 14, seven, six, and nine vessels, respectively). With a single exception, the CSF caused a marked constriction of the arterioles (Figs. 1 and 2), the maximum response occurring 1 to 4 minutes after the perivascular microinjection. The time course of changes in the caliber of two arterioles is shown in Fig. 3. The individual vasoconstrictive responses of the complete series of experiments are shown in Fig. 2. The absolute values are demonstrated in Table 3. The extent of induced vasoconstriction increased significantly ($p < 0.01$) with decreasing initial vessel size (Fig. 2). There were no significant differences in constricting activity between the four specimens when the variation in resting vessel caliber was taken into account, that is, the relatively less constrictive responses to CSF from Patients B and D were probably due to the fact that relatively larger vessels were exposed to these samples.

In 24 of the 36 experiments, the effect of perivascular application of a nifedipine solution (20 $\mu\text{g}/\text{ml}$) was tested when the CSF-induced vasoconstriction had reached a plateau. A marked dilatation was consistently seen, and its extent varied inversely with the resting (preconstricted) caliber of the vessel (Fig. 4). The duration of dilatation was, however, shorter in arterioles previously exposed to post-hemorrhagic patient CSF than in normal vessels of comparable size. Thus, at 10 minutes after the microapplication, the mean remaining dilatation in 14 observations was 14.2% (ranging from a constriction of 2.8% to a 41.8%

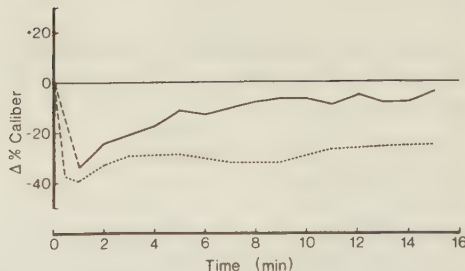


FIG. 3. Time course of changes in the caliber of two pial arterioles resulting from the perivascular application of CSF from Patient B (see Table 1). Unbroken line represents an arteriole with a resting caliber of 199 μm ; dotted line represents an arteriole with a resting caliber of 84.4 μm .

dilatation) as compared to 40.1% dilatation (ranging between 31.0% and 49.2% dilatation) in seven normal vessels of comparable size.

Discussion

The present study shows that ventricular CSF from three patients who had delayed cerebral ischemic deficits secondary to a ruptured aneurysm, and lumbar CSF from one patient who was asymptomatic but with pronounced angiographic vasospasm, each induced marked vasoconstriction in 35 out of 36 perivascular microinjections. By contrast, the application of mock or control human CSF either had no vascular effect or caused mild vasodilatation. Therefore, it is clear that CSF from patients with SAH can induce constriction of small cerebral arteries and arterioles *in vivo* in the cat.

There are two reports dealing with *in vivo* data on the subject of vasoconstricting agents in the CSF. Raynor, *et al.*,²² showed that topically applied serotonin could constrict exposed cortical arteries in cats, but did not obtain a reaction to topically applied CSF obtained from three patients with SAH. Boullin⁶ described experiments in two monkeys in which intracisternal injections of CSF from patients with delayed arterial spasm and ischemia had deleterious effects.

The vasoconstriction induced by CSF reached its peak 1 to 4 minutes after perivascular application. A similar temporal profile of a gradually increasing constriction in response to human post-hemorrhagic CSF has been observed in segments of human major cerebral arteries^{2,7} and we have also seen it in human pial arterioles *in vitro* (unpublished data). This time course is at variance with that seen after direct application of monoamines (for instance, serotonin).² Although this does not exclude the possibility that the effects of post-hemorrhagic CSF are mediated via vasoactive mono-

Vasoconstrictive response to human CSF in cats

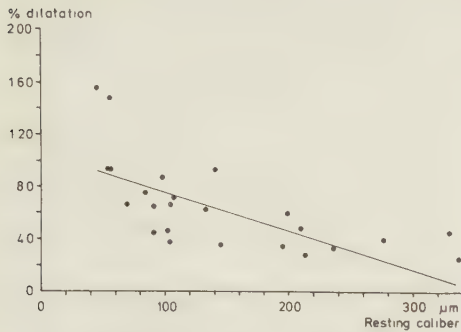


FIG. 4. The effect of the perivascular application of nifedipine (20 µg/ml) on CSF-constricted arterioles expressed as a percentage deviation from resting vessel caliber. Note the more pronounced dilatation with decreasing vessel size. The slope of the regression line is significantly different from zero ($p < 0.001$). Equation for the regression line: $Y = A + B \cdot x$.

amines, we have found in other *in vitro* experiments that the contraction induced by CSF is not blocked by specific serotonin and noradrenaline antagonists (unpublished data).

It is difficult to quantify the vasoconstrictive response of the patient CSF samples, since mock CSF_{A,B,D} and control CSF_{E,F} samples caused vasodilatation. A similar dilating effect with mock CSF has recently been reported by Crawford, *et al.*,¹⁰ using the same experimental model. The reason for this vasodilatation is not clear, but ionic differences between artificial CSF and the extracellular fluid of the cat cortical surface may be a contributing factor. The effect is not simply one of pH, because mock CSF_C and CSF_D were slightly alkaline (Table 2).

Both for vasoconstriction and for dilatation, smaller vessels were more reactive (Fig. 2). This has also been noted in experiments concerning the effects of perivascular application of calcium-free mock CSF⁴ and nifedipine.⁸ Among the possible explanations are: a higher vascular tone in distal resistance vessels;⁴ a greater sensitivity of distal resistance vessels to blockade of accessible extracellular calcium ions;⁸ or simply variability in diffusion characteristics resulting in limited access of the drug in the larger vessels.

In a previous study using this model, we demonstrated that perivascular microapplication of nifedipine resolved the *acute* vasoconstriction produced by perivascular bleeding.⁸ In the present experiments, the vasoconstriction induced by post-hemorrhagic CSF from humans with evidence of *delayed* vasospasm was also abolished by nifedipine. Even if the mechanisms behind the two types of experimentally induced vasoconstriction may differ, the prompt and con-

TABLE 3
Absolute vessel diameter (µm) before and after application of post-hemorrhage CSF and percentage change

Sample	Resting Diameter (µm)	Maximum Constriction Diameter (µm)	Percent Constriction
CSF _A	44.8	30.0	33.0
	54.2	32.4	40.3
	73.3	57.4	21.7
	84.4	51.2	39.3
	93.0	80.6	13.2
	101.5	49.9	50.8
	103.3	82.7	19.9
	109.7	65.8	40.0
	132.6	105.5	20.4
	144.8	93.3	35.6
	194.7	162.9	16.3
	199.1	132.2	33.6
	213.2	170.2	20.2
	276.0	232.8	15.7
CSF _B	55.8	47.9	14.0
	90.5	71.3	21.2
	97.5	76.6	21.4
	104.0	71.8	31.0
	140.1	111.9	20.1
	195.0	162.6	16.6
	206.4	172.3	16.5
CSF _C	235.9	205.7	12.8
	55.9	33.9	39.3
	60.9	46.6	23.5
	61.7	42.5	31.1
	90.2	53.6	40.6
CSF _D	100.7	80.9	19.7
	198.5	164.6	17.1
	55.3	31.8	42.5
	69.0	54.6	20.8
	83.4	66.3	20.5
	106.7	72.0	32.5
	188.4	159.3	15.4
	209.7	168.9	19.5
CSF _E	226.6	224.0	1.0
	336.7	246.0	26.9

sistent relaxation response of nifedipine is in agreement with the assumption that this drug interferes with a final common pathway for vascular smooth-muscle contraction.¹³

The fact that CSF from patients with a ruptured aneurysm contracts cerebral resistance vessels *in vivo* may be relevant to the pathogenesis and treatment of delayed cerebral ischemia secondary to SAH. Further studies, including testing of CSF collected serially after SAH and comparison of the responses with clinical events and angiographic findings, are warranted.

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VASOCONSTRICTOR ACTIVITY IN CEREBROSPINAL FLUID FROM PATIENTS
SUBJECTED TO EARLY SURGERY FOR RUPTURED INTRACRANIAL ANEURYSMS

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Bengt Ljunggren, M.D. and Philip Tagari, B.A.

SUMMARY

CSF vasoconstrictor activity was examined in serial samples obtained from 10 patients undergoing aneurysm clipping within 48 hours after SAH. There was no close relationship between vasoconstrictor activity in postoperative CSF samples and clinical condition or angiographic vasospasm. The identity of the vasoconstrictor substance/s in CSF was not established, but serotonin, histamine, norepinephrine, epinephrine, acetylcholine, or angiotensin_{II} were eliminated as prime vasoconstrictor agents inducing cerebral vasospasm. Differences in the temporal profile of the responses of isolated tissues to CSF from early and late surgery patients suggested that differing substances were involved in the production of spasm. A correlation between CSF potassium concentrations of vasoactive substances was found, but potassium could not account for vasoconstrictor activity of CSF. A log: linear correlation between total vasoconstrictor activity and total CSF collected could not be explained. Also, because of possible differences in the identity of vasoactive substances in CSF in this study compared to earlier studies, clinical comparisons based on apparent differences in pharmacological potency of CSF were not warranted. Nevertheless, apparent removal of subarachnoid blood by cisternal rinsing seemed to be a useful surgical adjunct.

INTRODUCTION

Subarachnoid blood is an essential factor in the etiology of the cerebral vasospasm syndrome, a complication frequently occurring after aneurysm rupture;⁶ its presence has been linked with the appearance of vasoconstrictor substances in the cerebrospinal fluid (CSF).^{1,2,5,7} Earlier work has shown that in some patients undergoing conventional late aneurysm surgery after subarachnoid hemorrhage (SAH), pharmacological activity of CSF seemed to be related to neurological deficits. Deteriorating neurological condition and the onset of angiographically demonstrated cerebral arterial spasm could be related to increasing concentrations of vasoconstrictor activity in the CSF.^{1,2}

Recently, early surgery (aneurysm clipping within 48 hours post-SAH) has been introduced, with emphasis on the removal of subarachnoid clots and saline rinsing of blood from the basal cisterns,^{10,19,20} in an attempt to break the chain of events leading to the appearance of vasoconstrictor substances in the CSF, and the onset of vasospasm.

The aims of the current study of SAH patients undergoing early surgery were two fold. Firstly, to investigate the presence of vasoconstrictor substances in the CSF on a daily basis, and to relate them to changes in neurological state and the onset of angiographic spasm. Secondly, to compare the concentrations of vasoconstrictor material present in the CSF with those found in previous studies with late surgery patients.^{1,7}

METHODS

Patients

The patients were 7 females and 3 males who suffered aneurysm rupture and were elected for early surgery within 48 hours post-SAH (for criteria, see Ref. 10). All patients were neurological grade III (Hunt and Hess,⁸ 1968) or less prior to surgery.

CSF collection

CSF was collected from the 10 patients either by lumbar puncture (8 patients) or from the lateral ventricle in two cases in whom ventricular fluid pressure was monitored according to Lundberg's technique¹¹ and CSF drained according to the regimen described by Sundbärg and Pontén.¹⁵ In 4 cases cisternal CSF was collected during surgery by placing a catheter with its tip into the opened basal cisterns prior to aneurysm dissection and clipping. Blood-contaminated cisternal CSF was collected by gentle aspiration and meticulous care was taken so as to avoid any contamination with blood from surgery.

The day of aneurysm rupture was designated day 0. Pre- and postoperative CSF samples were collected from day 0 to day 18.

All CSF was immediately frozen at -20°C and was not centrifuged. Samples remained frozen until assayed for vasoconstrictor activity as described below.

Estimation of vasoconstrictor activity

0.5 ml aliquots of CSF were bioassayed on either the rat stomach fundus³ or the human isolated basilar or vertebral artery⁴ suspended in oxygenated Krebs solution (pH 7.4) in a 5 ml organ bath warmed to 37°C . Muscle contractions were recorded isotonically with a Harvard transducer (Model No: 386) attached to a potentiometric chart recorder. Because the identity of the vasoconstrictor substances present in CSF is not determined, responses were quantitated by comparison with contractions produced by the prostaglandin PGE_2 .

Potassium

Potassium (K^+) was measured in each CSF sample by flame emission photometry on a Pye Unicam SP90A spectrometer using 20 μl aliquots (Technical Bulletin K2, 1969, Pye Unicam Ltd., Cambridge, England).

Pharmacological evaluation of the identity of vasoconstrictor substances in CSF

The compounds itemised in Table 3 were added sequentially in an attempt to reverse an established and sustained CSF-in-

TABLE 1: Summary of clinical data for patients without delayed cerebral ischaemic dysfunction (Group A)

Patient	Days after SAH	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Comments
Case 2 Male 54 ICA	Grade Spasm Vasoactivity K ⁺									NS										Discharged day 22 No neurological deficits other than cranial nerve palsy (pre-op damage)
Case 3 Female 37 ICA	Grade Spasm Vasoactivity K ⁺																			Discharged day 13 No deficits
Case 5 Female 53 ICA	Grade Spasm Vasoactivity K ⁺																			Discharged day 12 No deficits
Case 8 Male 45 ACoA	Grade Spasm Vasoactivity K ⁺																			Discharged day 16 No deficits
Case 9 Male 30 ACoA	Grade Spasm Vasoactivity K ⁺																			Postoperative angiogram demonstrated incomplete aneurysm occlusion. Re-op day 24 with complete occlusion. Discharged day 36. No deficits

NS, No angiographic spasm; DS, discrete angiographic spasm; MS, moderate angiographic spasm; SS, severe angiographic spasm; DC, discharged; *, surgery; RH, repeat SAH.

Vasoactivity refers to the vasoconstrictor response of the rat stomach fundus strip to 0.5 ml aliquots of CSF collected on the days indicated. The vasoconstrictor activity is expressed in terms of PGE₂ equivalents (nmols/l). Potassium (K⁺) concentrations in CSF are expressed in nmols/l.

TABLE 2: Summary of clinical data for patients with delayed cerebral ischaemic dysfunction (Group B)

Patient	Days after SAH	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Comments
Case 1 Female 69 MCA	Grade Spasm Vasoactivity K ⁺	II NS 8 *	II NS 8 *	III NS 8 *	IV NS 8 *	II NS 8 *	III NS 8 *	III NS 8 *	IV NS 8 *	IV NS 8 *	V NS 8 *	V NS 8 *	V NS 8 *	V NS 8 *	V NS 8 *	V NS 8 *	V NS 8 *	V NS 8 *	V NS 8 *	Angiography demonstrated significant intracerebral hematoma (4 x 3 x 3 cm) in addition to ruptured aneurysm. Second surgery with decompressive temporal lobe resection day 4. Ad mortem day 12
Case 4 Female 65 ICA	Grade Spasm Vasoactivity K ⁺	II NS 7 *	II NS 7 *	III NS 7 *	IV NS 7 *	III NS 7 *	III NS 7 *	III NS 7 *	IV NS 7 *	IV NS 7 *	IV NS 7 *	IV NS 7 *	IV NS 7 *	IV NS 7 *	IV NS 7 *	IV NS 7 *	IV NS 7 *	IV NS 7 *	IV NS 7 *	Repeat SAH day 7. Surgery day 8. Discharged day 32. Fully recovered after 3 months apart from oculomotor palsy sustained at first SAH
Case 6 Female 30 MCA	Grade Spasm Vasoactivity K ⁺	II NS 7 *	II NS 7 *	II NS 7 *	II NS 7 *	II NS 7 *	II NS 7 *	II NS 7 *	II NS 7 *	II NS 7 *	II NS 7 *	II NS 7 *	II NS 7 *	II NS 7 *	II NS 7 *	II NS 7 *	II NS 7 *	II NS 7 *	II NS 7 *	Fully mobilized when severe motor deficits from the operated hemisphere appeared on day (11)-12 postSAH. Discharged day 28. Recovered at later follow-up.
Case 7 Female 46 ICA	Grade Spasm Vasoactivity K ⁺	III NS 2 *	III NS 2 *	III NS 2 *	III NS 2 *	III NS 2 *	III NS 2 *	III NS 2 *	III NS 2 *	III NS 2 *	III NS 2 *	III NS 2 *	III NS 2 *	III NS 2 *	III NS 2 *	III NS 2 *	III NS 2 *	III NS 2 *	III NS 2 *	Arterial hypertension prior to SAH. Focal DID first appeared on day 8 post-SAH. At follow-up fully recovered from focal DID.
Case 10 Female 49 ICA	Grade Spasm Vasoactivity K ⁺	III NS 58 *	III NS 58 *	III NS 58 *	III NS 58 *	III NS 58 *	III NS 58 *	III NS 58 *	III NS 58 *	III NS 58 *	III NS 58 *	III NS 58 *	III NS 58 *	III NS 58 *	III NS 58 *	III NS 58 *	III NS 58 *	III NS 58 *	III NS 58 *	Rapid deterioration from day 9. Ad mortem day 13 from progressive severe DID.

NS, no angiographic spasm; MS, moderate angiographic spasm; SS, severe angiographic spasm; RH, repeat SAH; *, surgery; †, ad mortem. Vasoactivity refers to the vasoconstrictor response of the rat stomach fundus strip to 0.5 ml aliquots of CSF collected on the days indicated. The vasoconstrictor activity is expressed in terms of PGE₂ equivalents (nmols/l). Potassium (K⁺) concentrations in CSF are expressed in mmols/l.

duced contraction. All substances were dissolved in distilled water and applied to the tissues as described in Results. These pharmacological antagonists were obtained from the following sources: methysergide (Sandoz), mepyramine (May & Baker), phenoxybenzamine (Smith, Kline & French), propranolol (ICI Pharmaceuticals), atropine (Sigma), sarcosine¹-alanine⁸-angiotensin_{II} (Saralasin, Beckman).

RESULTS

Clinical information

Brief clinical details of the patients (neurological classification in Grades according to the system of Hunt & Hess⁸) are outlined in Tables 1 and 2.

The patients were divided into two groups based on the criterion of the presence or absence of delayed deficits of possible ischemic origin (DID). Group A consisted of 5 cases without signs of DID (Table 1) and Group B consisted of 5 cases who showed clinical deterioration with DID at some stage (Table 2).

Four vessel angiography was performed on all cases prior to surgery i.e. within 12-36 hours post-SAH. In the case of repeat hemorrhages angiography was performed on day 9 in case 8 and repeat carotid angiography on day 8 in case 4. In agreement with several earlier studies,^{9,12,13,16-18} no early spasm of major intracranial arteries was observed.

In 4 of the Group A patients postoperative angiography on days 6 to 8 post-SAH revealed no or minimal vasospasm (discreet angiographic spasm, Table 1). In the fifth patient (case 3) angiography on day 10 post-SAH showed severe vasospasm, although the patient remained free from neurological deficits.

In the Group B patients postoperative angiography demonstrated severe spasm in two cases (Nos. 6 and 10), on days 12 and 8, respectively, and moderate spasm on day 8 in one case (No. 7). In case 4 angiography did not show spasm one day after a repeat SAH (day 8). One patient had an associated intracerebral hematoma, in itself requiring urgent evacuation;

Fig.1. Vasoconstrictor activity of CSF obtained from case 3 1 to 6 days after SAH. 0.5 ml aliquots of CSF were bioassayed on the human isolated basilar artery (o-o) or the rat stomach fundus strip (●-●). Data from 3 assays on the rat tissue and 1 assay on the human tissue are shown. Ordinate: vasoconstrictor activity expressed as PGE_2 equivalents (nmol/l); Abscissa: time after SAH (days).

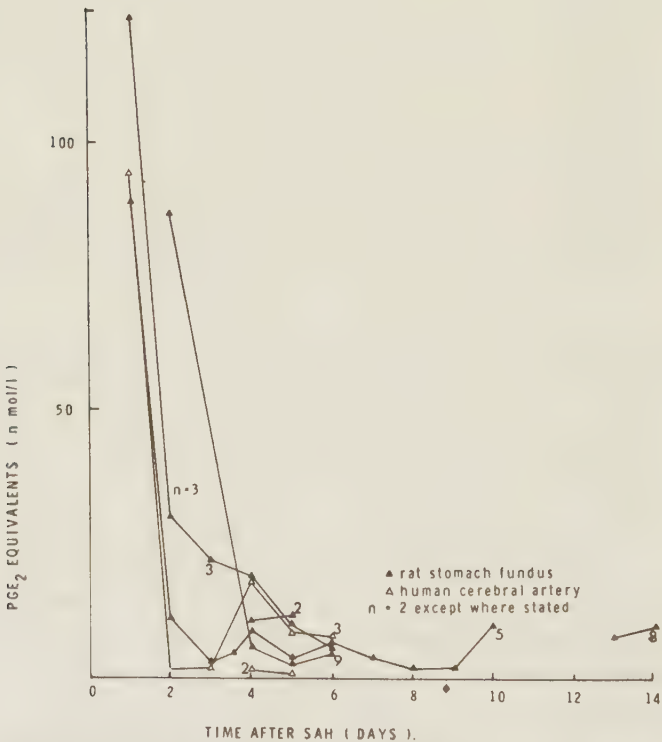
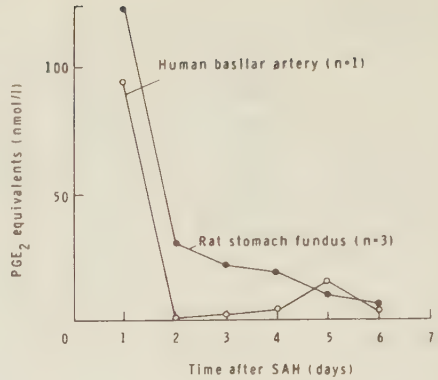


Fig.2 Vasoconstrictor activity in CSF from SAH patients undergoing early surgery without delayed ischemic deficits (Group A). Day 0= day of SAH. Each point is the mean value obtained from 2 bioassays on separate rat stomach fundus (▲) or human cerebral artery (△) preparations, except for case 3 where 3 bioassays were performed. Ordinate: vasoconstrictor activity (PGE_2 units nmol/l). Abscissa: Time after SAH

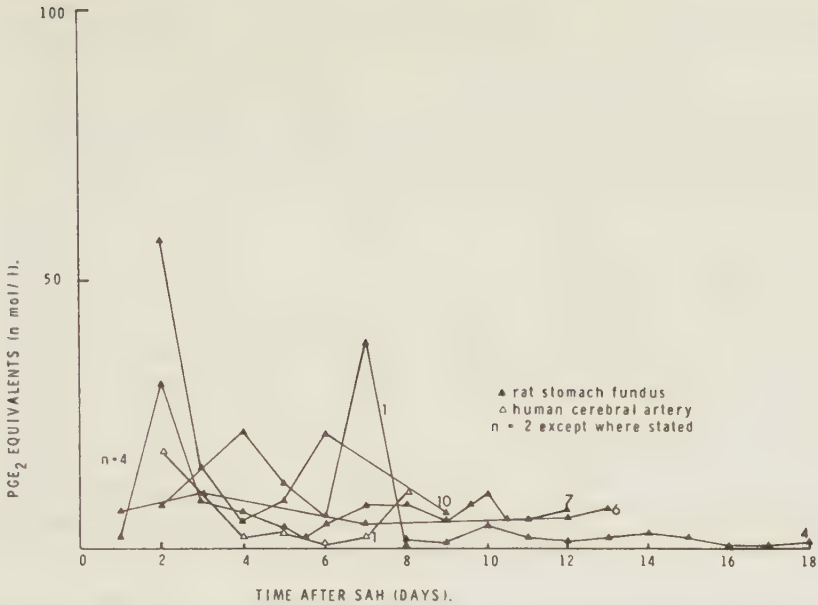


Fig.3 Vasoconstrictor activity in CSF from SAH patients undergoing early surgery with delayed ischemic deficits (Group B). Day 0= day of SAH. Each point is the mean value from 2 bioassays on separate rat stomach (▲) or human cerebral artery (△) preparations except for case 1 which was the mean of 4 bioassays. Ordinate: Vasoconstrictor activity (PGE₂ units nmol/l). Abscissa: Time after SAH (days).

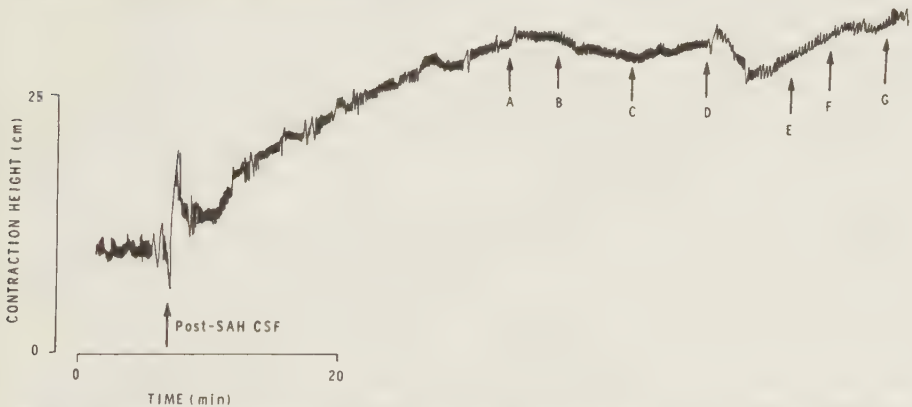


Fig.4 Attempted reversal of CSF induced contractions of rat stomach by specific pharmacological antagonists. At the arrow 0.5 ml of CSF was applied to the rat stomach fundus. The sample had a contractile activity equivalent to 11 nmols/l PGE₂. At the arrows labelled with letters the antagonists given in table 3 were added in the following order: A, Saralasin; B, Methysergide; C, Atropine; D and E, Mepyramine; F, Propranolol; G, Phenoxybenzamine. The concentrations used are given in Table 3. Ordinate: Contraction height (cm); abscissa: Time (min). Results of a single experiment are shown.

no postoperative angiography was done in this case (No. 1).

Two patients (cases 1 and 10) died 12 and 13 days after SAH; cases 6 and 7 had neurological deficits on discharge but recovered later. Cases 2 and 4 recovered except for cranial nerve palsy which both patients had sustained at the time of aneurysm rupture.

Pharmacological data

Due to difficulties in obtaining human cerebral arteries, not all samples were assayed on both the rat stomach fundus and human artery, but comparable activity has been observed upon both tissues. Figure 1 illustrates the comparative bioassays made with CSF from case 3.

Table 1 and Fig. 2 show the values for concentrations of vasoconstrictor materials in individual CSF samples from Group A patients in relation to neurological grade⁸ and time after SAH. While the values for vasoconstrictor activity were generally high shortly after SAH, and then declined, there was considerable variation and no close correlation with neurological state.

Table 2 and Fig. 3 present the same information relating to the Group B patients (with DID). Again there was no close correlation between the pharmacological activity of the CSF and the clinical state of the patient. The last values for vasoconstrictor activity (obtained 4 days prior to death) were low in the two patients (cases 1 and 10) who died of severe progressive cerebral ischemia.

The identities of the vasoconstrictor substances which appear in CSF after aneurysm rupture have not yet been established. Accordingly, a variety of pharmacological antagonists (Table 3) were used in attempts to reverse fully developed contractions of the isolated rat stomach fundus induced by post-SAH CSF (Fig. 4). The compounds itemised in Table 3 had no effects when added to the organ bath in the concentrations stated in the table. These concentrations of antagonists had been previously shown to have no contractile or relaxant effect in the absence of CSF. Furthermore they completely abolished contractions caused by the appropriate agonists given in doses

TABLE 3: Pharmacological antagonists which failed to reverse contractions produced by CSF from SAH patients

Antagonist	Concentration nmols/l	Specific drug effect (agonist) blocked
Methysergide	2000	Serotonin (5-HT)
Mepyramine	1000	Histamine
Phenoxybenzamine	1000	Noradrenaline (α -actions)
Propranolol	3000	Noradrenaline (β -actions)
Atropine	340	Acetylcholine
Sarcosine ¹ -alanine ⁸ -angiotensin _{II}	20	Angiotensin _{II}

The above results were obtained using the rat stomach fundus preparation (see 3). All agonists (right column) are potent vasoconstrictor agents on this tissue.

TABLE 4: Total volume of CSF, source of CSF, total vasoconstrictor activity and the (log) total vasoconstrictor activity of CSF collected from SAH patients.

Case	Total Vol. CSF (ml)	Total Vasoconstrictor Activity (p mols)	(log) total Vasoconstrictor Activity	Source of Post-op. CSF
Group A				
2	31	356	2.55	L
3	132	3606	3.58	L
5	135	1311	3.12	L
8	38	165	2.22	L
9	36	512	2.71	L
Mean $\pm$ S.E. 74.4 $\pm$ 24.1		1190 $\pm$ 635		
Group A				
1	142	1815	3.26	L
4	491	965	2.99	V
6	52	323	2.51	L
7	850	6930	3.84	V
10	68	1280	3.11	L
Mean $\pm$ S.E. 320.6 $\pm$ 154.5		2262 $\pm$ 1191		

Correlation between the (log) total vasoconstrictor activity and the total volume of CSF collected per patient:

GROUP A $R = 0.865$ $p < 0.05$

GROUP B $R = 0.75$ $p < 0.2$

ALL PATIENTS

(A + B) $R = 0.65$ $P < 0.05$

L, CSF sampled by lumbar puncture.

V, CSF sampled by ventricular drain.

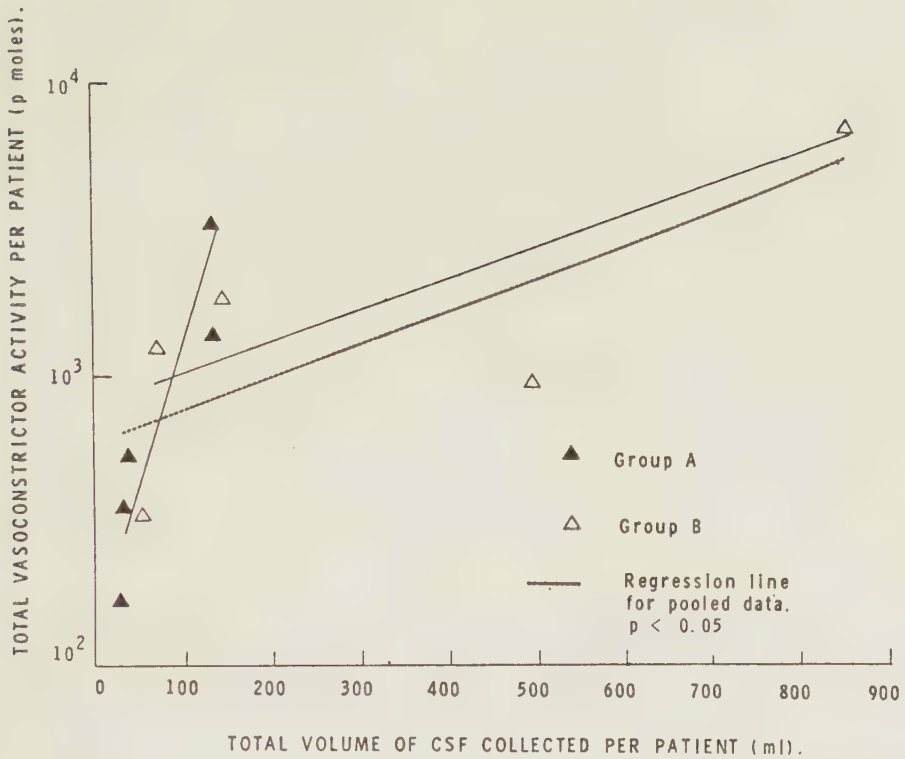


Fig. 5 Relationship between total vasoconstrictor activity in CSF (log pmols ordinate) and total volume of CSF collected per patient (ml abscissa). Computer plotted regression lines for groups A, B and A+B patient groups are shown. The correlation coefficients for Group A and Group A+B are significant at the 5% level (Table 4).

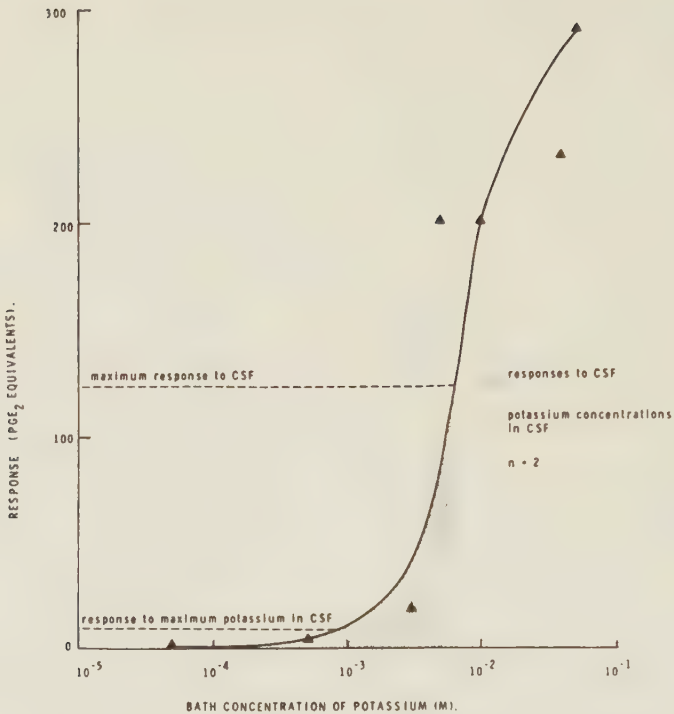


Fig.6 Dose response relationship for potassium-induced contractions of rat stomach fundus. Ordinate: Tissue response in PGE₂ equivalents (nmols/l). Abscissa: Potassium concentration in the organ bath (nmols/l). Values based on 2 determinations. The maximum response to CSF (upper dotted line) and the response produced by the maximum concentration of K⁺ detected in any CSF sample (9 nmols/l, case 7, lower dotted line) are indicated.

sufficient to elicit responses comparable to those produced by the most active CSF samples.

In contrast to earlier studies,^{1,7} substantial volumes of CSF were collected in the current investigation. Table 4 shows the total individual volumes of CSF available for bioassay and the total amount of vasoconstrictor material present (calculated by multiplying the concentration of activity by the volume collected on a given day). Both the mean volume of CSF and the mean total vasoactivity were greater in Group B (with DID) than in Group A. Furthermore there appeared to be a log: linear relationship between the total CSF volume and the total vasoconstrictor activity as indicated in Table 4 and Fig. 5. Correlation between these relationships was high and significant for Group A, lower and significant for the total number but not significant for Group B (Table 4).

Potassium

It was considered possible that the contractions were caused by potassium (K^+), although K^+ had been eliminated previously as a major vasoconstrictor in CSF from later surgery patients.^{4,14}

The K^+ concentrations in the CSF were in the same general range for both groups of patients (1-5 mmol/l) and never exceeded 10 mmol/l (Tables 1 and 2). Figure 6 shows the dose-response relationship for K^+ on the rat stomach fundus preparation.

Most importantly, the maximum K^+ concentration in any CSF sample (9 mmol/l) produced only a very small response, equivalent to 7.3% of the maximum response produced by CSF. Accordingly, CSF responses were not caused by K^+ . However, there was a highly significant correlation between K^+ concentration and vasoconstrictor activity in the CSF (Table 5).

Time course of CSF-induced contractile responses

Although there is no positive information as to the identity of the vasoconstrictor substances in CSF, there was indirect evidence of the involvement of different substances because there was a difference in the time course of responses

TABLE 5: The correlation between potassium concentration in post-SAH CSF and its vasoactivity

Case	Correlation Co-eff	P
Group A		
2	- (only 2 samples)	
3	0.83	<0.05
5	0.78	<0.005
8	- (only 2 samples)	
9	0.998	<0.01
Pooled	<u>0.748</u>	<0.0001
Group B		
1	0.27	<0.7
4	0.063	<0.9
6	0.841	<0.1
7	0.274	<0.4
10	0.31	<0.6
Pooled	<u>0.505</u>	<0.005
Total data pooled	<u>0.568</u>	<0.0001

The calculations shown are based on data presented in Figs. 2 and 3 for vasoconstrictor activity and Figs. 5 and 6 for K^+ concentrations in individual CSF samples.

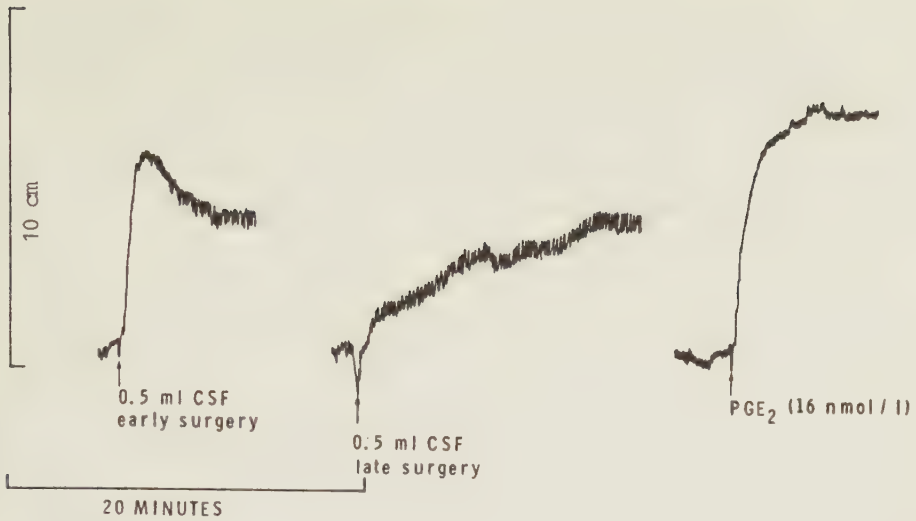


Fig. 7 Comparative responses to CSF collected from SAH patients undergoing early and late surgery. Left, contractile effect of CSF from case 5 in the present study. Centre, contractile response of CSF from a male, aged 21, collected 34 days after SAH at the time of surgery. This patient came from another unpublished study (Constant and Boullin). Right, response to 16 nmol/l PGE₂. Ordinate: Contractile response, height cm; abscissa: Time, 20 minutes.

of both the human basilar artery and the rat stomach fundus, to CSF from early versus late surgery cases. On both tissues CSF from cases of early surgery produced more rapid contractions. Fig. 7 shows the responses of the rat stomach fundus to samples of CSF taken from one patient in this study (early surgery) and from another patient who underwent surgery 34 days after SAH (see also Ref. 4).

DISCUSSION

Two previous studies of CSF vasoconstrictor activity, in relation to time after SAH and clinical condition, have been made.^{1,7} The first investigation examined CSF for up to 5 days after surgery which was carried out 5-10 days after aneurysm rupture.^{1,2} In 3 out of 20 cases high concentrations of vasoconstrictor activity in CSF were associated with neurological deficits and angiographically-proven cerebral vasospasm. The second study was performed over an extended period of 7 weeks; only isolated CSF samples were obtained from any given patient during this period⁷ so that correlations between CSF vasoactivity and neurological deficits were not possible. Surgery was conventionally timed, not earlier than 7 days after SAH. In the current investigation all cases had early surgery (no later than 48 hours after aneurysm rupture) combined with attempts at removal of as much subarachnoid blood as possible from the basal cisterns.

The presence of DID is commonly associated with the cerebral vasospasm syndrome. Thus it was expected that Group B patients (with DID) would have the highest values for vasoconstrictor activity in the CSF but this was not the case. In fact, the highest values for vasoconstrictor activity in this study were seen in 3 patients of Group A very soon after SAH and with a rapid decline by day 4 and were neither associated with constriction of the major cerebral arteries, visualised by angiography, nor with cerebral ischemic dysfunction. High concentrations of vasoconstrictor material immediately after aneurysm rupture did not lead to delayed ischemic deficits,

and there did not appear to be any clear relationship between CSF vasoactivity and clinical state, at least in this study.

In order to relate the pharmacological activity of CSF to neurological state, it is necessary to establish the identity of the substance or substances causing arterial constriction, and also the sensitivity of the arterial vessels in situ to these substances. While we have no data upon the latter, we have some information upon the former.

First, the differing temporal profiles of responses of the rat stomach fundus (Fig. 7) and the human basilar artery elicited by CSF from early and late surgery cases suggest that the timing of surgery, and the operative procedures, may have a profound effect on both the quantities and the natures of the vasoconstrictor substances released into the CSF after SAH.

Second, potassium (K^+) was eliminated as a major vasoconstrictor agent in this study, although there was a highly significant correlation between K^+ concentrations in CSF samples, and the concentration of vasoconstrictor materials (Table 5). This might be explained by a concomitant release of K^+ and vasoconstrictor materials from tissues damaged by subarachnoid blood.

Third, our investigation with specific pharmacological antagonists eliminated serotonin, epinephrine, norepinephrine, histamine, acetylcholine and angiotensin_{II} as the contractile agents appearing in the CSF after aneurysm rupture in both early and late surgery.

Thus there is no positive information as to the identity of the chemicals causing cerebral vasospasm in any of our three investigations.

The concentrations of unidentified vasoconstrictor material in the present series, up to 125 nmol/l prostaglandin E_2 equivalents were, in general, considerably lower, compared with earlier studies, where values as high as 25000^I, and from 150-2500⁷ nmol/l PGE_2 were recorded.

However, the clinical significance of these differences in concentrations of unknown substances cannot be established. Indeed standardisation of activity in terms of PGE_2 may give a false impression of potency without information upon the

sensitivity of the human cerebral arteries in situ. Nevertheless subarachnoid blood is an essential feature of the cerebral vasospasm syndrome. Accordingly our operative procedures with early drainage of blood-contaminated CSF from the basal cisterns, reduce the concentrations of vasoconstrictor substances in the cerebrovascular bed after subarachnoid hemorrhage and, hence, lessen the possibility of the appearance of cerebral vasospasm.

The significance of the unexpected correlation between (log) total vasoconstrictor activity and the total volume of CSF collected for assay from each patient is also not obvious. It might be explained by concentration gradients of vasoconstrictor substances descending from the basal cisterns through the lateral ventricles to the lumbar subarachnoid space. Such gradients have been described for dopamine metabolites (Erbart et al, 1980). Thus, drainage of larger volumes of CSF give an increasing contribution of cisternal CSF with higher concentrations of vasoconstrictors (supported by the high values found in pre-operative cisternal CSF samples). This implies that postoperative CSF drainage by repeated lumbar puncture or ventricular drain might reduce vasoconstrictor activity in the basal perivascular spaces. However, not all the CSF drained was collected (especially blood-contaminated CSF from surgery which was discarded). Furthermore, the data does not appear to show a reduction in the total amounts of vasoconstrictor material within the subarachnoid space. Thus postoperative drainage could only be recommended on the basis of the relationship between volume of CSF collected and total vasoconstrictor activity (derived from the data from the present series of ten patients) if the existence of gradients of vasoconstrictor material in CSF were established and if the above phenomenon were better understood.

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INDIVIDUAL VARIATIONS IN RESPONSE OF HUMAN CEREBRAL
ARTERIOLES TO VASOACTIVE SUBSTANCES, HUMAN PLASMA, AND
CEREBROSPINAL FLUID FROM PATIENTS WITH ANEURYSMAL
SUBARACHNOID HEMORRHAGE

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SUMMARY

The capricious appearance of delayed cerebral vasospasm in some but not all patients with an aneurysmal subarachnoid hemorrhage was the incitement for studying the reactivity of human cerebral arterioles in vitro to various vasoactive agents (prostaglandin $F_{2\alpha}$, noradrenaline, serotonin, human plasma and cerebrospinal fluid from patients with aneurysmal subarachnoid hemorrhage). There was a marked variability between arterioles from different individuals. The finding of a markedly individual profile in terms of reactivity towards vasoactive substances emphasizes the importance of a human cerebral vessel wall factor in the pathogenesis of cerebral vasospasm.

INTRODUCTION

Subarachnoid hemorrhage (SAH) due to aneurysm rupture is frequently complicated by delayed cerebral arterial spasm and symptoms of brain ischemia.^{1,2} The underlying mechanisms have evaded elucidation and no explanation has been put forward to account for the fact that these detrimental consequences do not complicate the course of every patient suffering from rupture of an intracranial aneurysm.

Research efforts have largely focused on finding and identifying vasoconstrictor material in cerebrospinal fluid (CSF) from patients with aneurysmal SAH.^{1-9,11,13-18,20-21,23} Although vasoactive agents in the hemorrhagic CSF probably are of importance in the pathogenesis of delayed arterial spasm after aneurysmal SAH, a recent study failed to prove any evidence for increased vasoconstrictor activity in the CSF in patients developing clinical or angiographical evidence of delayed brain ischemia or vasospasm.⁸ These findings indicate that individual differences in the responsiveness of human cerebral arteries and arterioles may account for the fact that SAH is not uniformly accompanied by delayed vasoconstriction and cerebral ischemic dysfunction.

The aim of the present study was to evaluate the responses of human pial arterioles to vasoactive substances, human plasma and CSF from patients with aneurysmal SAH.

METHODS

Pial arterioles ($\approx 500 \mu\text{m}$ in diameter) were removed by sharp cutting of a rectangular piece of cortical tissue before decompressive lobectomy in 22 patients suffering from cerebral gliomas not infiltrating the cortex. Electrocoagulation was not used. The cortical sample was placed in cooled ($4 - 10^{\circ}\text{C}$) Krebs-Ringer solution with the following composition (mM): NaCl 119, KCl 4.6, CaCl_2 1.5, MgCl 1.2, NaHCO_3 20.0, NaH_2PO_4 1.2 and glucose 11.0. Within 10 min after removal the arteries were dissected, cut into 2-3 mm long segments, and mounted on rigid parallel prongs in an organ bath with the aid of an operating microscope. The organ bath contained

5 ml of the Krebs-Ringer buffer solution. A gas mixture of 95% O₂ and 5% CO₂ was continuously bubbled through the organ bath and the stock solution to provide oxygenation, mixing, and preservation of a pH of approximately 7.4. Temperature was maintained at 37°C. The vessel wall was subjected to a passive force of approximately 5 mN. During equilibration for 90 min the vessel was repeatedly rinsed. The isometric tension was measured by means of a mechanical force transducer (FT 03C) and recorded by a Grass polygraph (RPS 7C8).

After equilibration the reactivity of the vessel was tested by changing the organ bath to a Krebs solution containing 127 mM potassium causing maximum depolarization. Only preparations showing reproducible responses to potassium (less than 10% change of maximum amplitude) were used for further experiments. The maximum vasoconstriction to the various test substances was expressed as percent of the potassium-induced contractions. At the end of the experiment a potassium-induced contraction confirmed the persistent reactivity of the vessel.

The following vasoactive substances were tested: noradrenaline (10^{-5} M), serotonin (10^{-5} M), and prostaglandin F_{2α} (10^{-5} M). These concentrations were chosen on the basis of previously performed experiments showing that maximum responses were obtained at these concentrations. All agents were dissolved in physiological saline and added in aliquots of 50 μl to the organ bath. The concentrations given are the final concentrations in the organ bath. The CSF and plasma samples (see below) were added in aliquots of 200 μl.

PATIENT MATERIAL

Vessel donors

Relevant data concerning the patients from whom cortical pial arterioles were obtained are briefly outlined in Table 1. All vessels were removed under general anaesthesia. Before intubation patients were given 0.005 mg/kg b.wt. phentanyl for analgesia and 0.03 mg/kg b.wt. pancuronium (Pavulon^R). Thiopenthal sodium (Pentothal^R) was supplied in doses to achieve sleep and after intubation the patients received

TABLE 1

VESSEL DONORS

<u>CASE</u> <u>(No)</u>	<u>AGE</u> <u>(years)</u>	<u>SEX</u>	<u>TUMOUR LOCATION</u>	<u>MEDICAL TREATMENT AT</u> <u>TIME OF SURGERY</u>
1	33	F	R. Temporal	Betapred ^R 4 mg x 4 Glau ^R 0,25 g x 4
2	66	M	R. Frontal	Betapred ^R 4 mg x 4 Fenanto ^R 0,2 g x 2
3	52	F	L. Temporal	Betapred ^R 4 mg x 4
4	57	F	L. Temporal	Betapred ^R 4 mg x 4
5	55	M	R. Frontal	Dexametason ^R 4 mg x 4 Lehydan ^R 0,2 g x 2
6	48	F	R. Parietal	Dexametason ^R 4 mg x 4 Tegretol ^R 0,2 g x 3
7	55	F	R. Frontal	Betapred ^R 2 mg x 3 Fenanto ^R 0,1 g x 3
8 *	59	F	R. Temporal	Betapred ^R 4 mg x 4 Tegretol ^R 0,2 g x 3 Fenanto ^R 0,1 g x 3 Aldactone ^R 25 mg x 3
9	47	F	R. Frontal	Dexametason ^R 3 mg x 4 Tegretol ^R 0,2 g x 3
10 *	59	M	L. Temporal	Betapred ^R 4 mg x 4 Tegretol ^R 0,2 g x 3 Seloken ^R 0,1 g x 2
11	26	F	R. Frontal	Betapred ^R 4 mg x 4 Fenanto ^R 0,1 g x 3
12	50	M	R. Frontal	Betapred ^R 4 mg x 4 Fenanto ^R 0,2 g x 2
13	34	F	R. Temporal	Fenanto ^R 0,1 g x 3 Tegretol ^R 0,2 g x 3 Fenemal ^R 100 mg x 1 Premaspin ^R 500 mg x 3
14	46	M	L. Temporal	Betapred ^R 4 mg x 4
15	60	F	L. Frontal	Betapred ^R 4 mg x 4 Tegretol ^R 0,2 g x 3 Tagamet ^R 200 mg x 5
16	75	M	L. Frontal	0 Med.
17	24	M	L. Frontal	Dexametason ^R 2 mg x 3 Fenanto ^R 0,1 g x 3
18	46	F	R. Frontal	Dexametason ^R 4 mg x 4
19	65	F	L. Temporal	Dexametason ^R 4 mg x 6
20	55	M	R. Temporal	Betapred ^R 4 mg x 4 Fenanto ^R 0,1 g x 3
21	65	M	R. Frontal	Dexametason ^R 4 mg x 4 Hiprex ^R 1 g x 2
22	65	M	R. Temporal	Betapred ^R 4 mg x 4 Stesolid ^R 2,5 mg x 3 Apodorm ^R 1 x 1

Explanations(for Table 1):

Betapred ^R	=	Betamethasone
Glaupax ^R	=	Acetazolamide
Fenantoin ^R	=	Phenytoin
Lehydan ^R	=	Phenytoin
Dexametason ^R	=	Dexamethasone
Tegretol ^R	=	Carbamezepine
Aldactone ^R	=	Spironolactone
Seloken ^R	=	Metoprolol
Fenemal ^R	=	Phenobarbital
Premaspin ^R	=	Acetylsalicylic acid
Tagamet ^R	=	Cimetidine
Hiprex ^R	=	Methenamine hippurate
Stesolid ^R	=	Diazepam
Apodorm ^R	=	Nitrazepam

T A B L E 2

Age/Sex	Patient A	Patient B	Patient C *	Patient D *§	Patient E *§	Patient F
Aneurysm site	Bif-BA, 2 L PCeA	R ICA	L ICA	R MCA	L ICA	Bif-BA
Source of CSF	Ventricle	Ventricle	Lumbar theca	Lumbar theca	Ventricle	Ventricle
Interval between last SAH and CSF sampling	15 days	9 days	5 days	12 days	7 days	12 days [†]
Interval between last SAH and onset of signs of cerebral ischemia	6 days	6 days	No signs	12 days	Drowsy to stuporous and confused since first SAH. No focal DID. Finally recovered	10-11 days
Clinical condition at time of CSF collection	Drowsy; R hemiparesis; Tachycardia; Normal BP; Equal size of pupils; Normal pupillary light reflexes; Afebrile. T 38.5°C.	Semicomatose; L arm monoplegia; Normal BP; Equal size of pupils; Normal pupillary light reflexes; Afebrile.	No neurological deficits. Normal T, BP and PR.	Fully awake and well oriented. Severe L hemiparesis	Stuporous, no focal DID.	Comatose
Late angiogram	N.D.	N.D.	No spasm on day 8 post-SAH.	Pronounced spasm on day 12 post-SAH (Threadlike arterial narrowing)	No spasm day 7 post-SAH No 1 and 1 month post-SAH No 1, respectively	Severe bilateral vasospasm day 7 post-SAH.
Drug treatment	None	Betamethasone (Betapred [®]) 4 mg x 4 Lm. Phenytoin (Fenatoin [®]) 100 mg x 3	Phenytoin (Fenatoin [®]) 100 mg x 3	None	Betamethasone (Betapred [®]) 4 mg x 4 i.m. x 3 Phenytoin (Fenatoin [®]) 100 mg x 3	Ampicillin 1 g x 3
Additional comments	Had a first SAH 3 weeks earlier; Also had L frontoparietal AVM.	Had SAH 17 days earlier; This SAH occurred from intra-operative aneurysm rupture during clipping.	Had early surgery with clipping of aneurysm and peroperative rinsing of basal cisterns within 24 hours after rupture.	Had early surgery with clipping of aneurysm and peroperative rinsing of basal cisterns within 48 hours after rupture.	Had two SAH:s with 6 days interval. Had early surgery with clipping of aneurysm within 48 hours after second rupture	

* = Patient included in series reported in J Neurosurg 1981 (Ljunggren, Brandt, Kågström & Sundbårg).

§ = Patient included in series reported in J Neurosurg 1981 (Boulin, Brandt, Ljunggren & Tagari).

Abbreviations: R = right; L = left; Bif-BA = bifurcation basilar artery; PCeA = posterior cerebral artery; MCA = middle cerebral artery; AVM = arterio-venous malformation; PR = pulse rate; BP = blood pressure; T = temperature (rectal); N.D. = not done; DID = delayed cerebral ischemic deficits.

additional Pavulon in a dose of 4-5 mg. Anaesthesia was maintained by ventilation with a gas mixture of 60% N_2O_2 and 40% O_2 .

CSF donors

CSF was obtained from 6 patients suffering from SAH due to a ruptured intracranial aneurysm (Table 2). Immediately after sampling the CSF was divided into minor portions and frozen at $-20^{\circ}C$ and was not centrifuged. The samples (denoted CSF_A to CSF_F) remained frozen until tested as described below.

Plasma donors

'Standard' test plasma was obtained from a healthy male at one occasion and frozen at $-20^{\circ}C$ in small portions. Specimens remained frozen until tested. In addition vessels from 5 donors were tested for the response of autologous plasma.

RESULTS

Responses to potassium ($K^+ = 127$ mM)

The responses to potassium of the preparations from the 22 patients are given in Table 3. The mean of the preparations from each patient are shown. Vessel segments from a single patient showed little variation in response; between patients the contractile force produced ranged between 4.4 to 19.0 mN despite the vascular in situ diameter being similar.

Responses to amines

Noradrenaline ($10^{-5}M$) and serotonin ($10^{-5}M$) induced constriction in vessels from all donors except case 1; insignificant responses to serotonin were also seen in cases 6 and 21 (See Table 3). In these cases, even higher concentrations of the amines ($10^{-4}M$) did not cause any contractile response.

The responses to the amines varied markedly not only when compared with those induced by potassium but also between the two amines (Table 3).

The concentrations of noradrenaline and serotonin which were used should guarantee maximal responses as shown in figures 1a and 1b.

CASE	POTASSIUM	NORADRENALINE	5-HYDROXYTRYPTAMINE
	mN	%	%
1	9.2	0	0
2	12.0	70	50
3	11.6	41	47
4	7.2	35	17
5	8.9	30	38
6	9.2	9	2
7	10.4	59	56
8	17.0	38	47
9	8.5	8	70
10	5.8	10	72
11	6.4	78	60
12	4.5	44	62
13	19.2	22	44
14	11.0	36	15
15	7.9	64	81
16	4.4	42	68
17	8.0	12	37
18	10.2	47	29
19	12.7	50	88
20	10.8	72	-
21	11.0	20	0
22	7.5	24	7

Table 3. Contractile responses to potassium(127mM),noradrenaline(10^{-5} M), and 5-hydroxytryptamine(10^{-5} M) in isolated human pial arteries. The responses to potassium are given in mN; the contractions induced by the amines in per cent of that induced by potassium

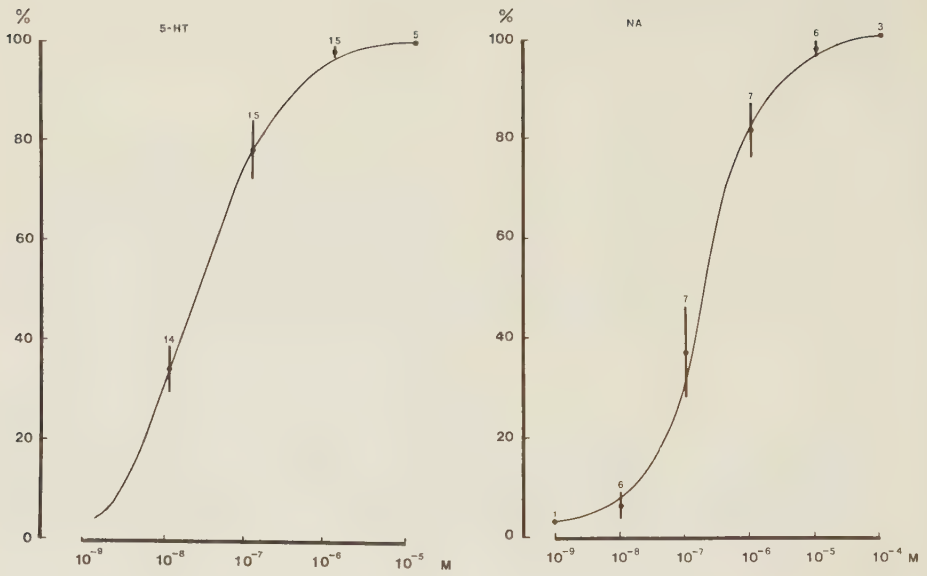


Fig. 1 a and b. Concentration - response curves for a) noradrenaline(NA) and b) 5-hydroxytryptamine (5-HT). Bars indicate mean values $\pm$ S.E.M.

Responses to prostaglandin $F_{2\alpha}$

Prostaglandin $F_{2\alpha}$ ($10^{-5}M$) was tested on cerebral arterioles from ten different vessel donors. As illustrated in Fig. 2 prostaglandin $F_{2\alpha}$ evoked marked constrictions of all investigated vessels, in two preparations even exceeding those induced by potassium ($K^+ = 127$ mM).

Responses to human plasma

The responses to the 'standard' plasma was tested on arterioles from 14 patients. As shown in Fig. 3 marked contractions were induced in the vessels from 7 of these donors. In case 10, plasma evoked a contractile response amounting to 95% of the response to potassium, exceeding the responses to both noradrenaline and serotonin (cf Table 3).

The vessels from 5 patients showed only insignificant responses to human plasma. Out of these 5 cases, four demonstrated an ordinary contractile response to noradrenaline and serotonin.

There were no obvious differences between the responses to standard plasma versus autologous plasma. Fresh blood was tested as for plasma in a few cases; no obvious difference in contractile response between blood and plasma was noted.

Responses to patient CSF

All the CSF samples induced constriction in vessels from one or several donors (Fig. 4). On the other hand, twelve vessels did not react to any CSF. The inconsistencies in responses were conspicuous. Although a marked variability in the CSF responses were noted between vessels from different donors, the pattern of individual vascular response to different CSF specimens was rather consistent (Fig. 4).

DISCUSSION

The present results indicate marked individual variations in the response of human cerebral arterioles to different vasoactive agents, plasma and hemorrhagic CSF, i.e. CSF from

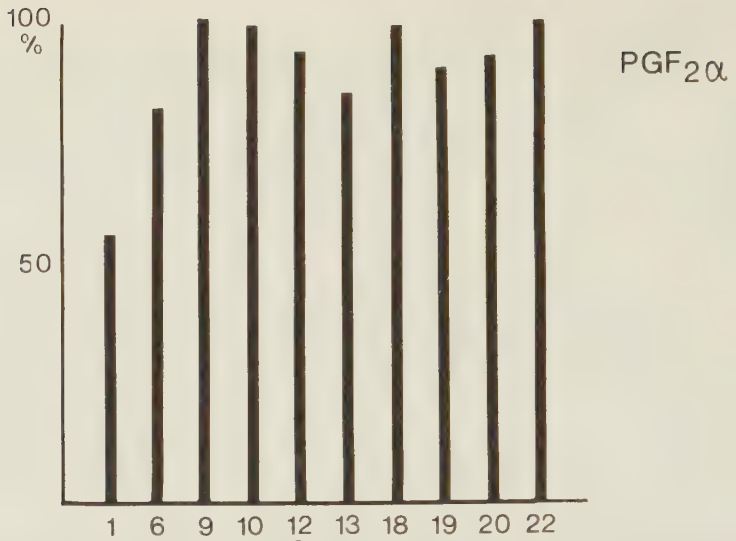


Fig.2 Prostaglandin F₂α-induced vasoconstrictor responses in vessels from the 10 donors tested. The responses are expressed as per cent of potassium-induced constrictions. The numbers refer to individual donors(see Table 1).

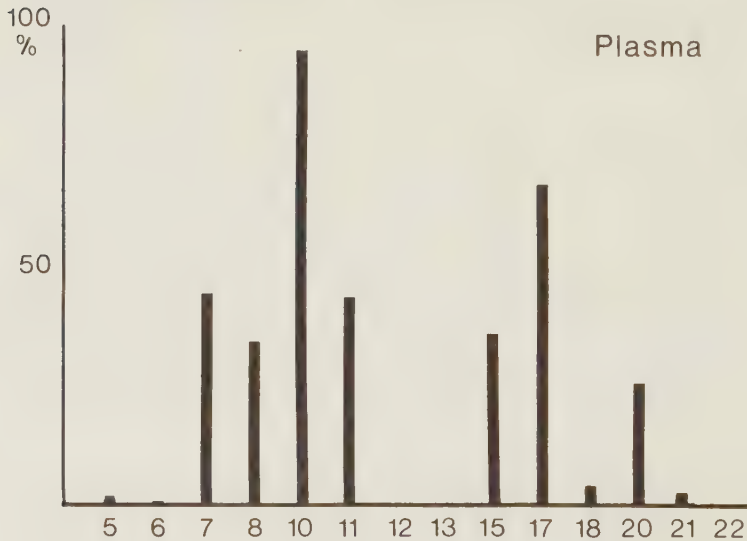


Fig.3 Plasma-induced vasoconstrictor responses on vessels from the 14 donors tested. The responses are expressed as per cent of potassium-induced constrictions.

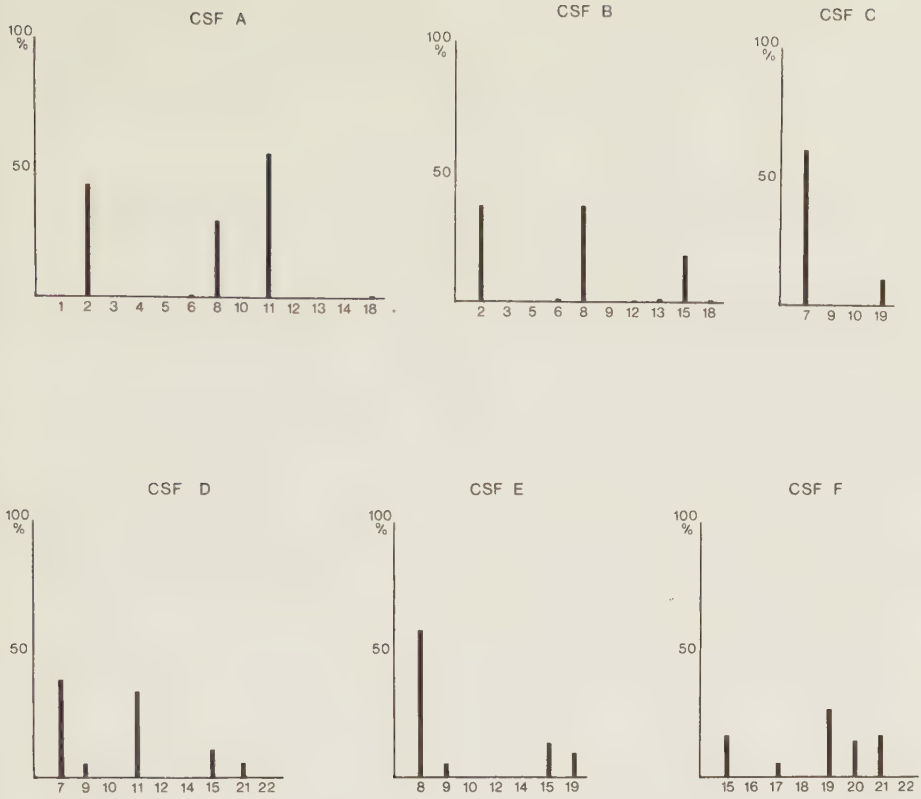


Fig. 4 The individual arteriolar responses to CSF_A - CSF_F in per cent of potassium-induced constrictions.

patients with aneurysmal SAH. Although the present findings are not at variance with those of others on canine² and feline cerebral arteries,¹⁶ such a marked variation in reactivity of human cerebral arterioles necessitates an analysis of possible contributing factors.

Differences in vascular responsiveness may exist between various cerebrovascular territories as well as between proximal and distal parts of the vascular system¹⁹ (for a more comprehensive review, see Vanhoutte, 1978²²). In the present study no obvious regional differences in vascular responsiveness were noted (cf Table 1 and results). All the tested vessels were almost equal in size. Consequently, it is hard to attribute the variability in vascular response to sampling artefacts.

The vessels were obtained from an obviously inhomogeneous group of patients (Table 1) - the neurosurgical intervention because of a malignant brain tumour and corticosteroid medication prior to surgery being the only features common to most patients (cases 13 and 16 did not receive steroid medication). Other factors such as age, sex and additional medication may have contributed to the variable results but none of these parameters was consistently correlated to a deviation in vascular response. Thus, it seems justified to conclude that human cerebral vessels in vitro exhibit a markedly individual profile in terms of reactivity towards vasoactive substances as exemplified in Fig. 5. Such a conclusion may have clinical implications provided that the in vitro findings do also apply to the in vivo situation. If so - the fact that various vessels reacted markedly different to the one and same CSF while the individual vascular reactions to various CSF samples were rather unanimous (Fig. 4), indicates a vascular factor as being important in the pathogenesis of arterial spasm after SAH. The marked individual variations in vascular responses to vasoactive substances in blood and hemorrhagic CSF may provide a part of the explanation for the fact that delayed arterial/arteriolar spasm does not uniformly occur after aneurysmal subarachnoid hemorrhage.

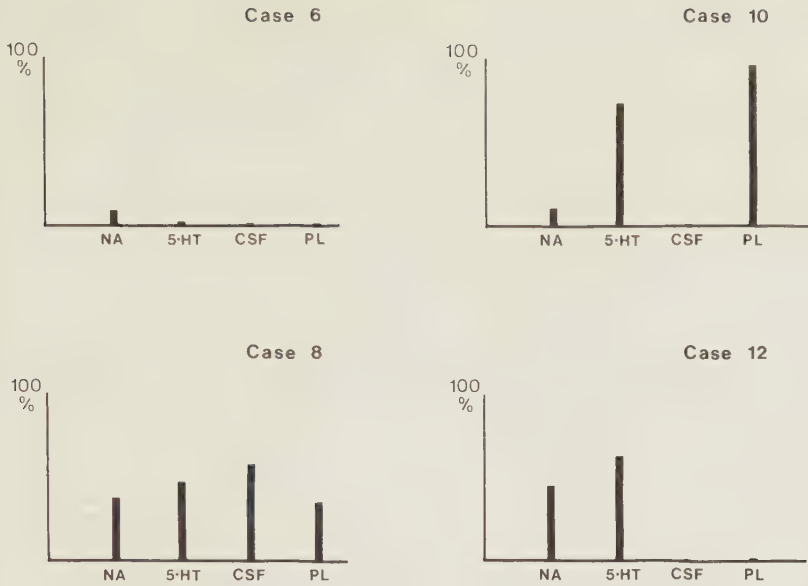


Fig. 5 The individual responses to noradrenaline, serotonin, CSF_A and plasma of arterioles from four different donors. Note the marked individual variations. Responses are expressed as per cent of potassium-induced constriction.

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EFFECTS OF INDOMETHACIN AND PROSTACYCLIN ON ISOLATED HUMAN PIAL
ARTERIES CONTRACTED BY CEREBROSPINAL FLUID FROM PATIENTS WITH
ANEURYSMAL SUBARACHNOID HEMORRHAGE

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SUMMARY

In small human cerebral arteries, pre-incubated with indomethacin, contractions induced by cerebrospinal fluid from subarachnoid hemorrhage patients were markedly increased. Also contractions induced by noradrenaline, but not 5-hydroxytryptamine, were augmented. Prostacyclin and its metabolite 6-keto-PGE₁ reversed the contractions induced by cerebrospinal fluid, as well as by noradrenaline, 5-hydroxytryptamine and prostaglandin F_{2α}. The findings suggest that these substances are able to counteract the influence of vasoconstrictor material in hemorrhagic cerebrospinal fluid. If the capacity to synthesize these 'protective' arachidonic acid metabolites is reduced, the resulting imbalance between contractile and relaxant forces acting on the vessel wall may lead to sustained cerebral vasoconstriction.

INTRODUCTION

It is well documented that cerebrospinal fluid (CSF) from patients with aneurysmal subarachnoid hemorrhage (SAH) may induce smooth muscle contraction.^{6,9,18} However, neither the development of delayed vasospasm of the large conducting cerebral arteries nor the development of delayed cerebral ischemic dysfunction is paralleled by an increase in vasoconstrictor activity in the CSF.⁸ Such activity has actually been shown to be highest in the early stage when vasospasm and ischemic symptoms have not yet developed.⁸ These findings indicate a disturbance of the protective mechanisms in the arterial wall maintaining normal cerebrovascular tone even in the presence of vasoconstrictor material in the perivascular space. This may be of major importance in the pathogenesis of delayed cerebral arterial spasm.

Prostacyclin (PGI_2), produced mainly in the vascular endothelium,²² is a potent dilator of pial vessels^{20,37,38} and a deficiency may be involved in the pathogenesis of cerebral vasospasm.^{5,7,33,34} PGI_2 is unstable at neutral pH in aqueous solution and is rapidly hydrolyzed to 6-keto-prostaglandin $\text{F}_{1\alpha}$ (6-keto-PGF $_{1\alpha}$) or metabolized to 6-keto-prostaglandin E_1 (6-keto-PGE $_1$).⁴³ The latter metabolite is relatively stable in aqueous solution and is a potent vasodilator.^{13,24,28} In fact, 6-keto-PGE $_1$ may be responsible for part of the activity ascribed to PGI_2 . 6-keto-PGF $_{1\alpha}$ which also is stable in aqueous solution is little vasoactive.²⁷

The objective of the present experiments was to clarify whether the effects of vasoconstrictor substances were modified by interference with the vascular prostaglandin synthesis. For this purpose, a human cerebral artery in vitro model was used.⁹ The effect of CSF, obtained from patients with an aneurysmal SAH, was examined before and after preincubation of the vessel with the prostaglandin synthetase inhibitor indomethacin.^{21-23, 32,42,44} Furthermore the effects of PGI_2 and 6-keto-PGE $_1$ were studied on vessels exposed to CSF from patients with an aneurysmal SAH.

METHODS

The procedure for obtaining human cerebral arteries and the in vitro testing of vascular responses have been described previously.⁹ Pial arteries (approximately 500 μm in diameter in situ) were removed by sharp cutting of a rectangular piece of cortical tissue before decompressive lobectomy was performed in 16 patients suffering from cerebral gliomas and in one case a subarachnoid hemorrhage with severe brain swelling. The brain cortex was in no case macroscopically tumorinfiltrated. Electrocoagulation was not used. The cortical samples were placed in cooled ($4 - 10^{\circ}\text{C}$) Krebs-Ringer solution with the following composition (mM concentration): NaCl 119, KCl 4.6, CaCl_2 1.5, MgCl 1.2, NaHCO_3 20.0, NaH_2PO_4 1.2 and glucose 11.0. Within 10 min after removal the arteries were dissected, cut into 2-3 mm long segments, and mounted on rigid parallel prongs in an organ bath with the aid of an operating microscope. The organ bath contained 5 ml of the Krebs-Ringer buffer solution. A gas mixture of 95% O_2 and 5% CO_2 was continuously bubbled through the organ bath and the stock solution to provide oxygenation, mixing, and preservation of a pH of approximately 7.4. Temperature was maintained at 37°C . The vessel wall was subjected to a passive force of approximately 5 mN. During equilibration for 90 min the vessel was repeatedly rinsed. The isometric tension was measured by means of a mechanical force transducer (Grass FT 03C) and recorded by a Grass polygraph (RPS 7C8).

After equilibration the reactivity of the vessel was tested by changing the organ bath to a Krebs solution containing 127 mM potassium causing maximum depolarization. Only preparations showing reproducible responses to potassium (less than 10% change of maximum amplitude) were used for further experiments. The vascular responses to CSF (see below), noradrenaline (NA) and 5-hydroxytryptamine (5-HT) were compared before and after exposing the vessels to drugs interfering with arachidonic acid metabolism. These included indomethacin, eicosatetraynoic acid (ETYA), and tranylcypromine at varying concentrations (see results).

Prostacyclin sodium salt (PGI_2) and 6-keto-PGE₁ (Upjohn, USA) were dissolved in acetone and dispensed in test tubes using Eppendorf adjustable micropipettes. The acetone was then rapidly evaporated under a stream of nitrogen leaving the pure prostaglandin in the tubes. These were stored at -20°C and the contents dissolved in ice-cold 0.05M TRIS-buffer solution (pH 10.4) within three hours before use. Dilutions of PGI_2 solutions were made up in the same solvent and kept on ice until added to the organ bath. 6-keto-PGE₁ solutions were further diluted with phosphate buffer at neutral pH and kept in the refrigerator until immediately before use. Stock solutions of prostaglandin $\text{F}_{2\alpha}$ (Amoglandin^R, Astra, Sweden) were further diluted with phosphate buffer.

NA and 5-HT were obtained from Sigma. The concentrations of the amines are given as concentrations of the free base in the organ bath. The concentrations of PGI_2 and 6-keto-PGE₁ are given as concentration of free acid.

The CSF samples (see below) were added in aliquots of 200 μl to the organ bath.

PATIENT MATERIAL

Vessel donors

The experiments were performed on 41 preparations from 16 patients. Relevant data concerning the patients from whom cortical pial arteries were obtained are briefly outlined in Table 1. All vessels were removed under general anaesthesia. Before intubation patients were given 0.005 mg/kg b.wt. fentanyl for analgesia and 0.03 mg/kg b.wt. Pancuronium (Pavulon^R). Thiopental (Pentothal^R) was supplied in doses to achieve sleep and after intubation the patients received additional Pavulon^R in a dose of 4-5 mg. Anaesthesia was maintained by ventilation with a gas mixture of 35% O_2 and 65% N_2O_2 .

<u>Case</u>	<u>Age</u> (years)	<u>Sex</u>	<u>Tumour location</u>	<u>Medical treatment at time</u> <u>of surgery</u>
1	24	M	L. Frontal	Dexacort ^R 2mg x 3 Fenanto ^R 0.1g x 3
2	46	F	R. Frontal	Dexacort ^R 4mg x 4
3	75	M	L. Frontal	0 med.
4	60	F	R. Temporal	Betapred ^R 4mg x 4 Tegretol ^R 0.2g x 3 Fenanto ^R 0.1g x 3 Aldactone ^R 25mg x 3
5	60	F	L. Frontal	Betapred ^R 4mg x 4
6	64	M	R. Temporal	Betapred ^R 4mg x 4
7	59	M	L. Temporal	Betapred ^R 4mg x 4
8	66	F	L. Temporal	Betapred ^R 4mg x 4 Fenanto ^R 0.1g x 3
9	65	M	R. Frontal	Betapred ^R 4mg x 4
10	55	M	R. Temporal	Betapred ^R 4mg x 4 Fenanto ^R 0.1g x 3
11	66	M	L. Temporal	Dexacort ^R 4mg x 4
12	63	F	L. Temporal	Betapred ^R 4mg x 4 Seloken 200mg x 1
13	65	M	L. Temporal	Betapred ^R 4mg x 4
14	65	F	L. Temporal	Dexacort ^R 4mg x 6
15	51	M	R. Frontal	Betapred ^R 4mg x 4 Fenanto ^R 0.2g x 2
16	57	F	SAH. R. Temporal	Epanutin ^R 250mg x 3 Betapred ^R 6mg x 4

Table 1. Vessel donors. Age and sex distribution, tumour localization, and medical treatment at time of surgery. Case 4 had arterial hypertension. Case 16 represents a patient with subarachnoid hemorrhage, and temporal lobe resection was performed due to brain swelling.

Explanations:

Fenanto^R = Phenytoin
Betapred^R = Betamethasone
Tegretol^R = Carbamazepine
Aldactone^R = Spironolactone

Tagamet^R = Cimetidine
Dexacort^R = Dexamethasone
Seloken^R = Metoprolol
Epanutin^R = Phenytoinnatrium

CSF Donors

T A B L E 2

Age/Sex	51/F	65/F	51/F
Aneurysm site	Bif-BA	L ICA	R ICA
Source of CSF	Ventricle	Ventricle	Ventricle
Interval between last SAH and CSF sampling	12 days	7 days	9 days
Interval between last SAH and onset of signs of cerebral ischemia	10-11 days	Drowsy to stuporous and confused since first SAH. No focal DID. Finally recovered.	6 days
Clinical condition at time of CSF collection	Comatose	Stuporous, no focal DID.	Semicomatose; L arm monoplegia; Normal PR and BP; equal size of pupils; Normal pupillary light reflexes; T 38.5°C.
Late angiogram	Severe bilateral vasospasm day 7 post-SAH	No spasm day 7 post-SAH (No 1) and one month post-SAH (No 1), respectively	N.D.
Drug treatment	Doktacillin 1g x 3	Betamethasone (Betapred ^R) 4 mg x 4 i.m. Phenytoin (Fenantoin ^R) 100 mg x 3	Betametasone (Betapred ^R) 4 mg x 4 i.m. Phenytoin (Fenantoin ^R) 100 mg x 3
Additional comments		Had two SAH:s with 6 days interval. Had early surgery with clipping of aneurysm and peroperative rinsing of basal cisterns within 48 hours after second rupture	Had SAH 17 days earlier; This SAH occurred from intraoperative aneurysm rupture during clipping

Abbreviations: R = right; L = left; Bif-BA = bifurcation basilar artery; ICA = internal carotid artery; BP = blood pressure.
 PR = pulse rate; T = temperature (rectal); ND = not done; DID = Delayed ischemic deficits.

CSF donors

CSF was obtained from 3 patients suffering from SAH due to a ruptured intracranial aneurysm (Table 2). Immediately after sampling the CSF was divided into minor portions and frozen at -20°C and was not centrifuged. The samples remained frozen until tested as described below.

RESULTS

In the 41 vessel preparations 127 mM potassium induced pronounced contractions ranging between 4.1 mN and 12.2 mN (mean 8.4 mN). The contractile effects of all other drugs were expressed as per cent of maximum potassium-induced vessel contraction.

Effects of CSF before and after pretreatment with indomethacin

In 9 preparations none of the three CSF specimens caused any significant response. In the remaining 22 preparations the CSF-induced contractions varied from 5 to 38% of the potassium-induced response (Fig. 1).

In 20 of 34 preparations indomethacin (10^{-6}M) per se induced contractions. Mean values for indomethacin-induced contractions are given in Fig. 1. These contractions developed slowly and maximum was reached after several minutes (Fig. 2).

After incubation with indomethacin the CSF-induced contractions were markedly augmented (Fig. 1) in 19 out of the 22 preparations. Before indomethacin incubation (10 min) the exposure to CSF was repeated at least twice and without exception the response to the last exposure never exceeded the previous (Fig. 2). In 5 of the 9 preparations where CSF did not induce any significant contraction, incubation with indomethacin resulted in a conspicuous contractile response to CSF. The effect of indomethacin per se was subtracted in the results given for CSF-induced contraction after pretreatment with indomethacin (Fig. 1). The enhancement was similar for the three different CSF specimens.

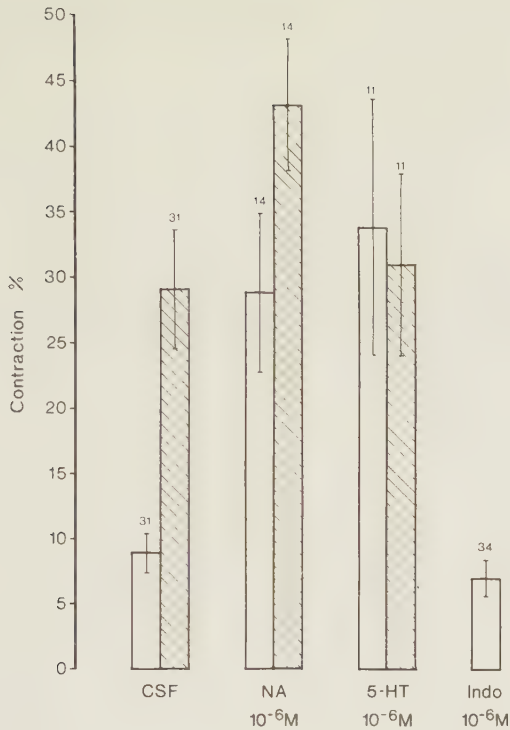


Fig. 1 Contractile vascular responses to cerebrospinal fluid(CSF), noradrenaline(NA), and 5-hydroxytryptamine(5-HT), before(unfilled columns) and after(broken columns) pretreatment with indomethacin. The vertical bars denote $\pm$ S.E.M. Number of experiments are indicated above columns. The differences before and after indomethacin pretreatment are significant for CSF ($p < 0.001$) and for NA ($p < 0.005$) (Students *t*-test). The effects of indomethacin *per se* (Indo) are also given. Contractions are expressed as per cent of response to potassium (127 mM) before addition of drugs.

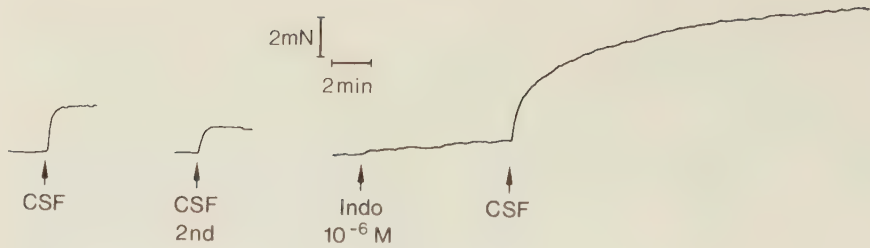


Fig. 2 Responses to cerebrospinal fluid (CSF) in an isolated human pial vessel preparation before and after indomethacin (Indo) pretreatment. Note the decrease in response to CSF at the second exposure.



Fig. 3 Relaxant effects of 6-keto-prostaglandin- E_1 (6-keto-PGE₁) on contractions induced by potassium (127 mM), 5-hydroxytryptamine (5-HT), noradrenaline (NA), and cerebrospinal fluid (CSF) after pretreatment with indomethacin (Indo).

Effects of 5-HT and NA before and after pretreatment with indomethacin

5-HT was tested on 11 preparations, nine of which showed a variable degree of contraction (from 5 to 89%) of the potassium-induced response. As shown in Fig. 1 the response to 5-HT was not influenced by preincubation with indomethacin. In the two preparations not responding to 5-HT, preincubation with indomethacin did not produce any contractile response to subsequent exposure to 5-HT.

NA was tested on 14 preparations before and after pretreatment with indomethacin. As for 5-HT there were individual variations in the responses. These ranged from 6 to 92% of the potassium-induced contraction. However, it was assured that in the one and same preparation the response to the amines did not change significantly at repeated exposures. In contrast to 5-HT, the NA-induced contractions were markedly augmented by pretreatment with indomethacin as shown in Fig.1.

Relaxant effects of PGI_2 and 6-keto-PGE₁

In preparations contracted by 127 mM potassium, 6-keto-PGE₁ had a weak and inconsistent relaxant effect (Fig. 3) whereas PGI_2 produced a small but consistent relaxation.

In vessels contracted by CSF or indomethacin + CSF both PGI_2 and 6-keto-PGE₁ invariably induced an immediate and pronounced relaxation. In fact, in most experiments the resulting relaxation was well below resting vessel tension (Fig. 3). To induce the same degree of relaxation with 6-keto-PGE₁ a tenfold higher concentration was needed than for PGI_2 .

In 5-HT and NA contracted preparations PGI_2 ($2.5 \cdot 10^{-7} \text{M}$) and 6-keto-PGE₁ ($3.0 \cdot 10^{-6} \text{M}$) had a relaxant effect similar to that seen in CSF-induced contractions (Figs. 3 and 4). The response to PGI_2 at a concentration of $2.5 \cdot 10^{-6} \text{M}$ was biphasic. After approximately 30 sec the initial relaxation was followed by a pronounced contraction which, in turn, was followed by a second, more slowly, though finally complete relaxation (Fig. 4).

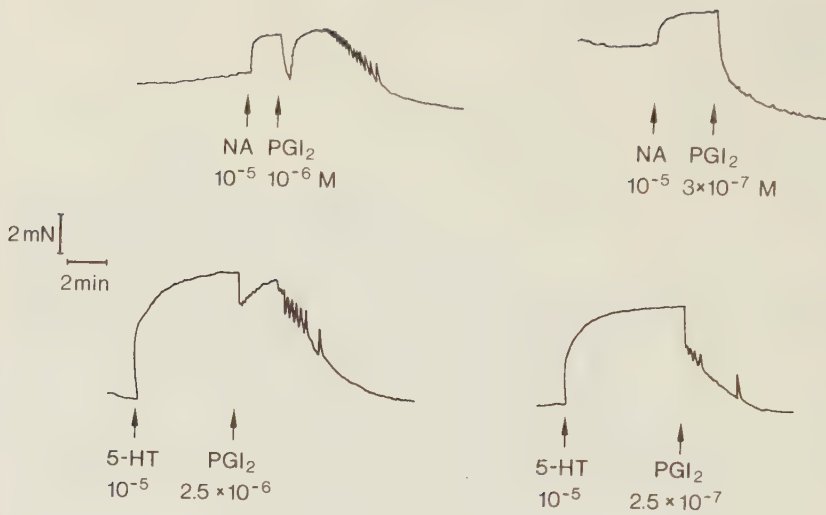
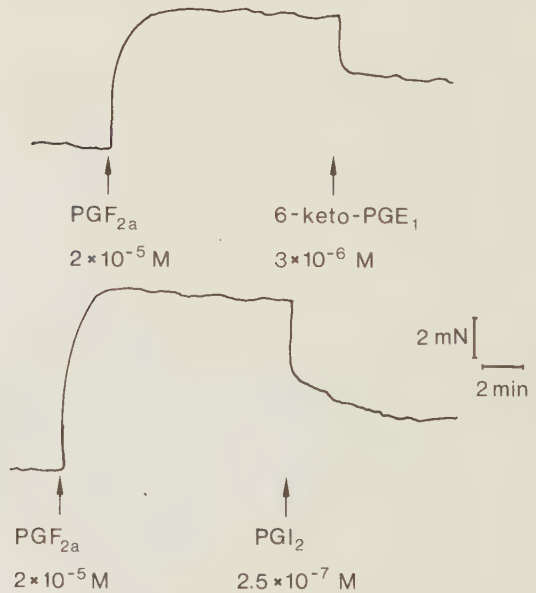


Fig. 4 Relaxant effects of prostacyclin (PGI₂) in two different concentrations on contractions induced by noradrenaline (NA) and 5-hydroxytryptamine (5-HT).

Fig. 5 Relaxant effects of prostacyclin (PGI₂) and 6-keto-PGE₁ on prostaglandin F_{2a}-induced contractions.



In preparations contracted by $\text{PGF}_{2\alpha}$ ($2.0 \cdot 10^{-5}\text{M}$) both PGI_2 and 6-keto- PGE_1 had a pronounced relaxant action ($\approx 50\%$ of $\text{PGF}_{2\alpha}$ -induced contraction. Fig. 5).

Effects of arachidonic acid and drugs interfering with prostaglandin synthesis

A few pilot experiments were performed with arachidonic acid (AA), its acetylenic analogue, eicosatetraynoic acid (ETYA), which inhibits both the lipoxygenase and cyclo-oxygenase pathways of the AA cascade, and tranylcypromine, which is said in vitro to block the formation of PGI_2 .⁴² All three substances induced concentration-dependent contractions per se. There were no obvious modifications of the CSF-induced responses after preincubation with ETYA ($3.3 \cdot 10^{-5}\text{M}$) or tranylcypromine ($10^{-6} - 10^{-4}\text{M}$). AA-induced ($3.3 \cdot 10^{-5}\text{M}$) contractions were not blocked by ETYA or indomethacin.

DISCUSSION

In animal experiments it has been shown that an intact prostaglandin synthesis maintains normal cerebrovascular tone and mediates the dilatatory response to hypercapnia^{14,26,31,35} whereas the increases in cerebral blood flow (CBF) in hypoxia,³¹ hypoglycemia,²⁵ and seizures¹⁹ are not mediated by mechanisms related to prostaglandin metabolism.

It was proposed that prostaglandin production in the cerebral arterial wall may be involved in the pathogenesis of delayed cerebral vasospasm,^{7,10-12,16,20} which complicates the course of many but not all patients suffering from rupture of an intracranial aneurysm.¹⁵

Experimental SAH in dogs and monkeys produces severe ultrastructural vessel changes which can be demonstrated 3 to 7 days after SAH.^{1-4,36} These changes consist of vacuoles and dense bodies in frequently detached endothelial cells, appearance of intimal hyperplasia, and platelet adherence. Alksne and Smith⁴ proposed the term vasonecrosis to this progressive vascular lesion. The morphological findings

indicate that the intima, where PGI_2 is synthesized, is specifically subjected to progressive injury after aneurysmal SAH. This may cause a decrease in PGI_2 synthesis and release which may result in a sustained contraction of the cerebral arteries and arterioles, as suggested by Sano and co-workers.^{5,33,34}

Indomethacin is a potent inhibitor of prostaglandin synthesis by blocking the enzyme cyclooxygenase which converts free arachidonic acid to unstable endoperoxides.^{21-23,42,44} PGI_2 , a main arachidonic acid metabolite in arteries, is formed mainly by the endothelial layer.^{17,21-23} It prevents platelet aggregation and relaxes arterial smooth muscle, including that of pial vessels.^{7,10,11,20,40}

Sakanashi and co-workers³² demonstrated a concentration-dependent contractile effect of indomethacin, in concentrations ranging from 10^{-8} to 10^{-5}M , on isolated dog coronary arterial strips; the effects were attributed to inhibition of the synthesis of vasodilating prostaglandins. Indomethacin in the concentration used in the present study, 10^{-6}M , which corresponds roughly to therapeutic concentrations observed in patients, seems to represent a threshold concentration in vitro for the contractile effects of the drug. On the other hand, high concentrations of indomethacin, 10^{-4} to 10^{-3}M , had relaxant effects as shown by Chapleau and co-workers on isolated dog basilar arteries.¹²

In a previous study it was shown that the immediate response of isolated human cerebral vessels to CSF from patients with aneurysmal SAH varied from no effect to marked contractions.⁹ As shown in the present study, contractions induced by CSF from SAH patients in indomethacin pretreated human cerebral arteries were markedly increased. Also contractions produced by NA but not 5-HT were augmented. The reasons for this difference between the amines are unclear. The fact that PGI_2 and its metabolite 6-keto- PGE_1 reversed the contractions induced by CSF from humans with aneurysmal SAH as well as by NA, 5-HT and $\text{PGF}_{2\alpha}$ suggest that these substances are able to counteract the influence of vasoconstrictor material in the CSF.

The biphasic response to high concentrations of PGI_2 with an initial relaxation followed by contraction and a final complete relaxation may be explained by the fact that PGI_2 can have both contractile and relaxant effects. It is well known that in high concentrations PGI_2 contracts vascular smooth muscle. On the other hand, in low concentrations the drug has a relaxant action which was confirmed in the present study. The biphasic response may therefore be a distribution phenomenon on addition of PGI_2 to the organ bath - the final complete relaxation being produced when part of the PGI_2 in the bath had been degraded.

The augmentation of CSF- and NA-induced contractions by indomethacin may be due to inhibition of synthesis of 'protective' arachidonic acid metabolites. One may speculate that the development of delayed cerebral vasospasm after aneurysmal SAH may be due to a local disturbance of the synthesis of prostacyclin and/or other dilatatory metabolites.

The identities of the vasoconstrictor substances which appear in CSF after aneurysmal rupture have not been established. However, 5-HT and NA do not seem to be major contributing agents.⁸ Urquilla suggested the involvement of the nucleotide uridine 5'-triphosphate (UTP) in cerebral vasospasm.³⁸ With reservation for species differences our results do not suggest UTP as a major contributing factor in CSF-induced vasoconstriction as UTP-induced contractions of isolated dog middle cerebral artery segments were not altered by preincubation with 10^{-5}M indomethacin.³⁸

The present preliminary experiments did not show that tranylcypromine had any obvious intensifying effect on CSF-induced contractions. This result is consonant with those of Rajar and de Gaetano,²⁹ who did not find an inhibitory activity of tranylcypromine on vascular PGI_2 synthesis in rats. ETYA blocked the effects neither of CSF nor of AA. However, the drug per se exhibited a marked concentration-dependent contractile action, which makes the consequences of possible inhibitory effects on lipooxygenase and cyclooxygenase activity difficult to evaluate.

Indomethacin has been suggested to afford an alternative treatment of cerebral vasoconstriction after SAH.^{16,27,41} The rationale behind this suggestion is that indomethacin may reduce an excessive synthesis of prostaglandins with constrictor properties and inhibit the formation of thromboxane. Our study only reflects the in vitro situation; yet, we think that clinical attempts at treatment of cerebral vasospasm with indomethacin should await further in vivo studies since the present results suggest that indomethacin might be harmful and promote spasm.

Subarachnoid hemorrhage and vasospasm is often accompanied by fever²⁹ and indomethacin, with its antipyretic properties, is frequently administered to patients suffering from SAH. With the background stated above it is suggested that patients with SAH and fever should not receive indomethacin.

ACKNOWLEDGEMENTS

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EFFECTS OF EXTRACELLULAR CALCIUM AND OF CALCIUM ANTAGONISTS
ON THE CONTRACTILE RESPONSES OF ISOLATED HUMAN PIAL AND MESEN-
TERIC ARTERIES

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SUMMARY

In isolated human pial arteries (diameter 0.4 - 0.5mm) contractions were produced by potassium, noradrenaline, serotonin and prostaglandin F_{2a} . For comparison, experiments were also performed on human mesenteric arteries. Threshold concentration for potassium induced contraction in pial arteries was about 10 mM; in mesenteric arteries it was 3-5 mM higher. In pial arteries the calcium antagonists nifedipine and nimodipine caused an almost complete relaxation of contractions induced by potassium at drug concentrations relaxing prostaglandin F_{2a} contracted vessels to only about 60 per cent. Both nifedipine and nimodipine effectively inhibited contraction elicited by noradrenaline and serotonin in pial arteries. Nifedipine had a higher potency for relaxing cerebral than mesenteric arteries contracted by potassium ($p < 0.001$). No such difference was demonstrated for nimodipine. In pial arteries pretreated in a calcium-free medium for 30 min. potassium depolarisation elicited contractions reaching a maximum amplitude of about 40 per cent of that evoked in normal Krebs solution. Both nifedipine and nimodipine effectively inhibited contractions induced by calcium in pial arteries pretreated in a calcium-free medium and depolarised by potassium. The results suggest that potassium, amines and prostaglandin F_{2a} activate isolated pial and mesenteric arteries by different calcium dependent mechanisms and confirm the potent relaxant effects of nifedipine and nimodipine in these vessels.

INTRODUCTION

Cerebral blood flow as well as the blood flow to other organs is regulated by changes in blood vessel diameter. These changes are mediated through a complex interaction between neurotransmitters, circulating and local hormones and different physicochemical factors (Johansson 1978). Even if important differences exist between the activation mechanisms of arteries in various vascular regions, many basic mechanisms seem to be common, and differences often depend upon different degrees of development of the mechanisms (Casteels 1980). A final pathway, common for all activation mechanisms, is an increase of the cytoplasmatic calcium concentration.

Johansson (1971) suggested that myogenic spontaneous tone and vasomotor responses to extrinsic stimuli in many blood vessels are mediated by electrical responses to the smooth muscle cell membrane, a process termed electromechanical coupling by Somlyo and Somlyo (1968). Electromechanical coupling has been demonstrated in isolated rabbit pial arteries (Lusomvuko et al 1979).

Action potentials initiating mechanical activity are in some types of smooth muscle caused by influx of calcium from the extracellular medium (Brading et al 1968). Bolton (1979) suggested that this calcium influx occurs through specific voltage sensitive calcium channels, which open when the potential across the membrane is reduced. The same type of channel may also be opened by e.g. potassium depolarisation. In many blood vessels, tension development may be produced without associated electrical activity (Somlyo and Somlyo 1968, Droogmans et al 1977) - pharmacomechanical coupling. In this case, the increase in cytoplasmatic calcium is believed to depend on the opening of another type of calcium channel in the membrane, the receptor operated channel (Bolton 1979). It cannot be excluded, however, that drugs activating smooth muscle by pharmacomechanical coupling, e.g. noradrenaline, will also induce a release of calcium from intracellular stores.

Although much information is available concerning calcium dependent activation mechanisms in vascular smooth muscle from

various regions, in animals as well as in man, little is known about these processes in human cerebral arteries. In the present study, isolated human pial arteries were activated by potassium, noradrenaline, serotonin and prostaglandin F_{2a} in normal Krebs solution, in the presence of drugs inhibiting calcium influx, and in a calcium-free medium. For comparison, data on isolated human mesenteric arteries were included.

METHODS

Blood vessels

Pial arteries diameter (approximately 0.5 mm in situ) were removed by sharp cutting of a rectangular piece of cortical tissue before decompressive lobectomy was performed in patients suffering from cerebral gliomas. The brain cortex was in no case tumour infiltrated. All vessels were removed under general anaesthesia. Before intubation patients were given 0.005 mg/kg b.wt. fentanyl for analgesia and 0.03 mg/kg b.wt. pancuronium (Pavulon^R). Thiopenthal sodium (Pentothal^R) was supplied in doses to achieve sleep and after intubation the patients received additional Pavulon^R in a dose of 4-5 mg. Anaesthesia was maintained by ventilation with a gas mixture of 60% N_2O_2 and 40% O_2 .

In patients undergoing abdominal surgery and bowel resection, macroscopically normal parts of the omentum were removed. Anaesthesia was given as described above.

All vessel specimens were placed in a chilled Krebs solution and transported to the laboratory for further dissection. Both pial and mesenteric vessels were often mounted in the organ bath within fifteen minutes after removal. Segments with an outer diameter of 0.2 - 0.5 mm and about 3 - 5 mm long were selected. Some vessels were kept in Krebs solution at 4°C for later use (up to 24 h).

Recording

The arteries were mounted in a 5-ml temperature-controlled

organ bath containing Krebs solution. The temperature was kept at 37°C and the bathing solution was bubbled with 95% O₂ and 5% CO₂ giving a pH of approximately 7.4. A system of L-formed metal holders was used for recording of the mechanical activity of the vessel segments (Högestätt et al 1980). Isometric tension was recorded by means of Grass Ft 03 transducers connected to a Grass P7 polygraph. After mounting, the vessel segments were subjected to a passive force of 5 mN and allowed to stabilize at this level during an equilibration period of 1½ h.

Assessment of effects

All preparations were contracted by potassium 127 mM as a test of reactivity of the vessels. Concentration-response curves were obtained for potassium, prostaglandin F_{2a} (PGF_{2a}), noradrenaline (NA) and 5-hydroxytryptamine (5-HT) by adding these contractile agents cumulatively. Potassium was also added non-cumulatively. The effects of calcium antagonists were recorded in two ways:

1) the ability of the drugs to relax preparations contracted by potassium 127 mM or PGF_{2a} 2.5 uM was assessed by adding them cumulatively. Their relaxant effect was expressed as per cent of the imposed contraction.

2) the ability to inhibit contractions was studied by adding the drugs 10 min before contractions were elicited by NA or 5-HT.

In order to study the effects of extracellular calcium on the action of the contractile and relaxant agents, the responses were investigated after a standard pre-treatment in a calcium-free medium (for composition, see below) for 30 min.

Solutions

The "standard Krebs" solution had the following composition (mM): NaCl 119, KCl 4.6, CaCl₂ 1.5, MgCl₂ 1.2, NaHCO₃ 20, NaH₂PO₄ 1.2, glucose 11. The solutions used for potassium-depolarisation contained 3, 10, 12.5, 15, 20, 40, 60, or 127 mM

KCl, and NaCl was decreased correspondingly. Otherwise the composition was the same as for standard Krebs solution. Calcium-free medium was prepared by omitting CaCl_2 and adding 0.01 mM EGTA to reduce the amount of contaminating calcium. All the solutions were prepared on the day of the experiment. Double-distilled water and analytical grade chemicals were employed.

Drugs

L-noradrenaline hydrochloride (Sigma), 5-hydroxytryptamine creatinine hydrochloride (Sigma), cocaine hydrochloride (ACO), prostaglandin F_{2a} (Amoglandin, Astra) and propranolol hydrochloride (ACO), were dissolved in 0,9% NaCl. Nifedipine (Bayer AG) and nimodipine (Bayer AG) were supplied in ampoules containing 0,1 mg/ml, kept in dark at 4°C and opened just before use. All concentrations throughout are given as the final concentration in the organ bath.

Analysis of data

The EC_{50} values (molar concentration of drug at which half maximum response occurs) were calculated and used as measures of potency. The results are generally expressed as mean values $\pm$ standard errors of the mean. Differences between mean values were determined by Student's t-test.

RESULTS

Effects of potassium

In both cerebral and mesenteric arteries potassium added cumulatively or non-cumulatively induced concentration-dependent contractile responses. No relaxant effects were observed. In cerebral arteries, the threshold for contraction was approximately 10 mM (fig 1) and more than half maximum effect was

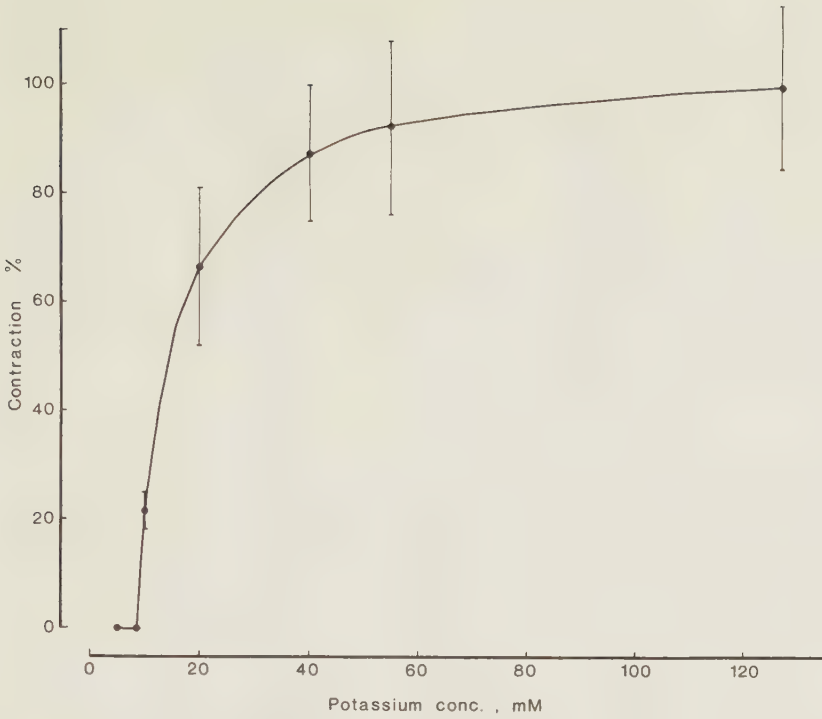


Fig.1 Concentration-response curve for potassium in human pial arteries. Bars indicate mean $\pm$ S.E.M.



Fig.2 Potassium (127 mM) induced contractions in two human pial arteries. Note the biphasic contractile pattern.

reached at a concentration of 20 mM. The response to potassium was unchanged whether or not cocaine 1 μ M and propranolol 1 μ M or prazosin 1 μ M were present in the buffer solution.

Contractions produced by 127 mM potassium consisting of one rapid and one slowly developing phase (fig 2), were reproducible for at least 5-6 h if the preparations were rapidly washed with normal Krebs solution when maximum tension was reached, and if an equilibration period of 20-30 min was allowed between the stimulations. A contraction was stable for at least 30 min. Threshold for potassium contraction in mesenteric arteries was about 13-15 mM.

Effects of PGF_{2a} and amines

PGF_{2a} evoked a concentration-related contraction of the cerebral arteries. At a concentration of 2.5 μ M maximum tension was obtained being of similar magnitude as that produced by 127 mM potassium (fig 4). The response was reproducible for up to 5-6 h if elicited at 20-30 min intervals. A contraction was stable for at least 20-30 min. Mesenteric arteries, on the other hand, responded poorly to PGF_{2a} . When a contraction was induced, it was weak and failed to be stable.

NA and 5-HT both contracted cerebral (fig 5a,b) and mesenteric arteries in a concentration-related way. These responses were almost as reproducible and as stable as those elicited by PGF_{2a} in pial vessels. However, when compared to potassium-induced contractions on the same vessels, the maximum contraction induced by the amines varied.

Responses for vessels investigated immediately after removal were similar to those of vessels kept overnight at 4°C.

Effects of calcium-free medium

After 30 min exposure to a calcium-free medium the responses to potassium decreased from $7.5 \pm 0.6 \text{ mN}$ ($n=11$) to $2.4 \pm 0.2 \text{ mN}$, and those to NA and 5-HT from 2.3 ± 0.8 ($n=4$) and $3.1 \pm 0.5 \text{ mN}$ ($n=5$) to 0.9 ± 0.3 and $1.1 \pm 0.3 \text{ mN}$, respectively (Fig. 3). The contractions induced by potassium were completely restored 30 min af-

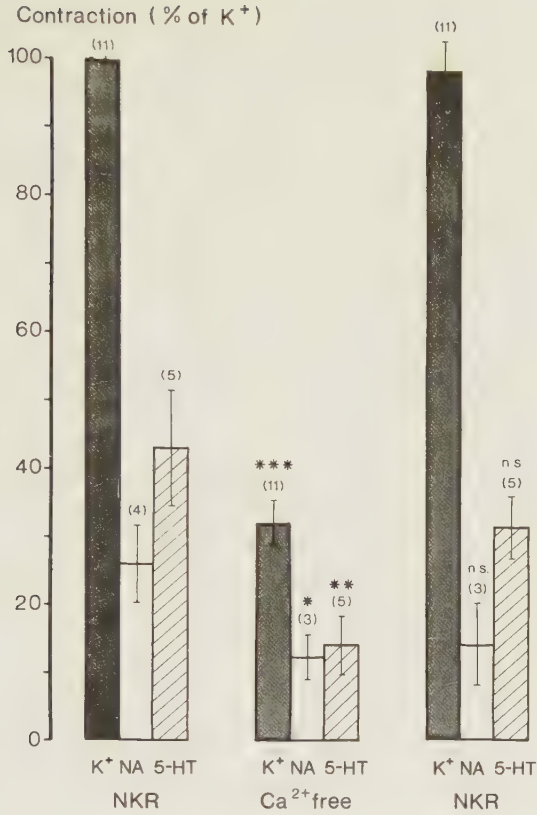


Fig.3 Contractile responses of human pial arteries to potassium(K^+ , 127mM), noradrenaline(NA, $10^{-5}M$), and 5-hydroxytryptamine(5-HT $10^{-5}M$) in normal Krebs(NKR) solution, after exposure for 30 min. in a calcium-free solution and 30 min. after reintroduction of calcium to the extracellular medium. Bars indicate mean values $\pm$ S.E.M. Numbers within brackets denote numbers of experiments. The degree of significance of change in response indicated above bars. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$



Fig. 4 Relaxant effects of nifedipine and nimodipine on contractions of human pial arteries induced by potassium (127 mM) and PGF_{2α} (10⁻⁵ M).

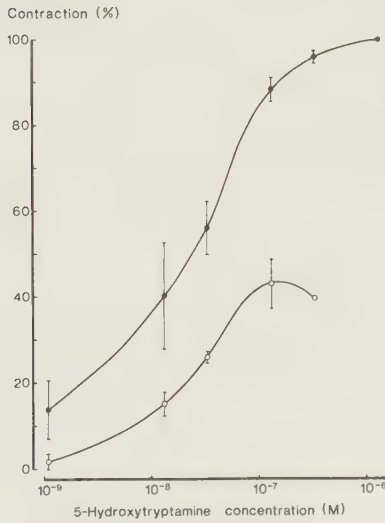
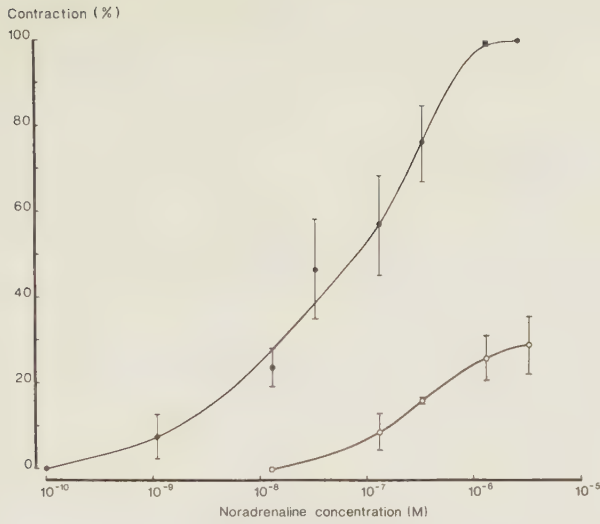


Fig. 5 Concentration-response curves for noradrenaline and 5-hydroxytryptamine in human pial arteries in absence (filled symbols) and presence (open symbols) of nifedipine (0.15 μ M). Bars indicate mean values $\pm$ S.E.M.

ter re-introduction of calcium into the bathing solution. (Fig. 3), whereas those evoked by NA and 5-HT were only partially restored.

Effects of calcium antagonists

The pial vessels contracted by potassium were almost completely relaxed (95%) by nifedipine and nimodipine, 3 μ M (Figs. 4, 7). In a concentration of 0.15 μ M nifedipine reduced the maximum contractions produced by NA to 29 (Fig. 5a) and those of 5-HT to 42 per cent (Fig. 5b) of the control contractions obtained by the amines in the absence of the calcium antagonists.

The contractions produced by potassium in human mesenteric arteries were almost completely inhibited (96%) by the calcium antagonists (Fig. 6).

Both pial and mesenteric vessels were thus relaxed to approximately the same extent, but small differences in EC_{50} -values could be demonstrated between the two vascular regions and between the calcium antagonists (Table 1). Nifedipine was more potent in cerebral than in mesenteric vessels ($p < 0.001$). Nimodipine had lower EC_{50} values than nifedipine in both types of vessel (Table 1). However, there was no statistically significant difference between cerebral and mesenteric arteries in response to nimodipine ($p > 0.05$).

Pial vessels contracted by $PGF_{2\alpha}$ were relaxed by both nifedipine and nimodipine to about 60 per cent at concentrations causing almost complete relaxation of potassium contracted vessels (Figs. 4, 7). There were no significant differences in EC_{50} values between the drugs. However, the EC_{50} values in $PGF_{2\alpha}$ contracted vessels were significantly higher for both drugs than in potassium contracted arteries ($p < 0.001$) (Table 1).

Inhibition of calcium induced contractions

When pre-treated for 30 min in a calcium-free solution and depolarized by 127 mM potassium the pial vessels were contracted in a concentration related way when the extracellular cal-

Fig.6 Concentration-response curves for the relaxant effects of nifedipine(circles) and nimodipine(triangles) on potassium-induced contractions in human mesenteric arteries. Bars indicate mean values $\pm$ S.E.M.

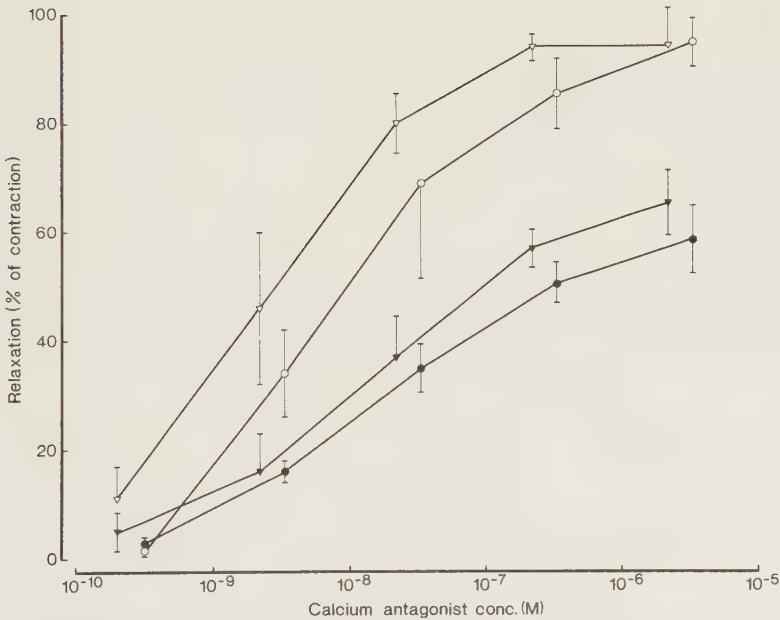
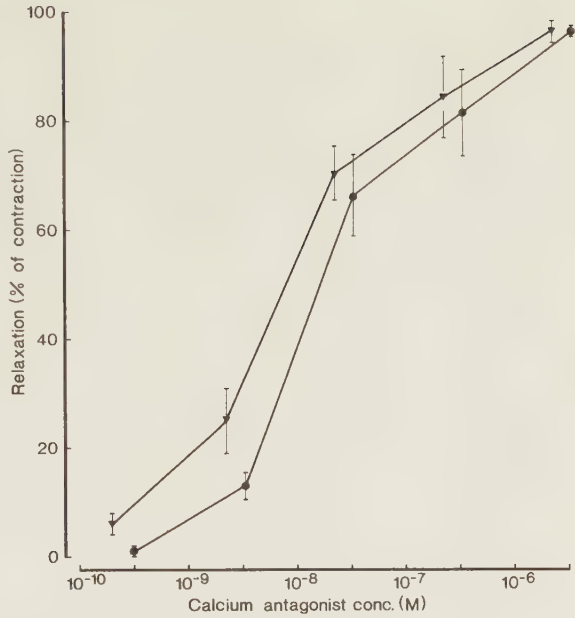


Fig.7 Concentration-response curves for the relaxant effects of nifedipine (circles) and nimodipine(triangles) on contractions in human pial arteries induced by potassium 127 mM(open symbols) and $\text{PGF}_{2\alpha}$ 10^{-5} M(filled symbols). Bars indicate mean values $\pm$ S.E.M.

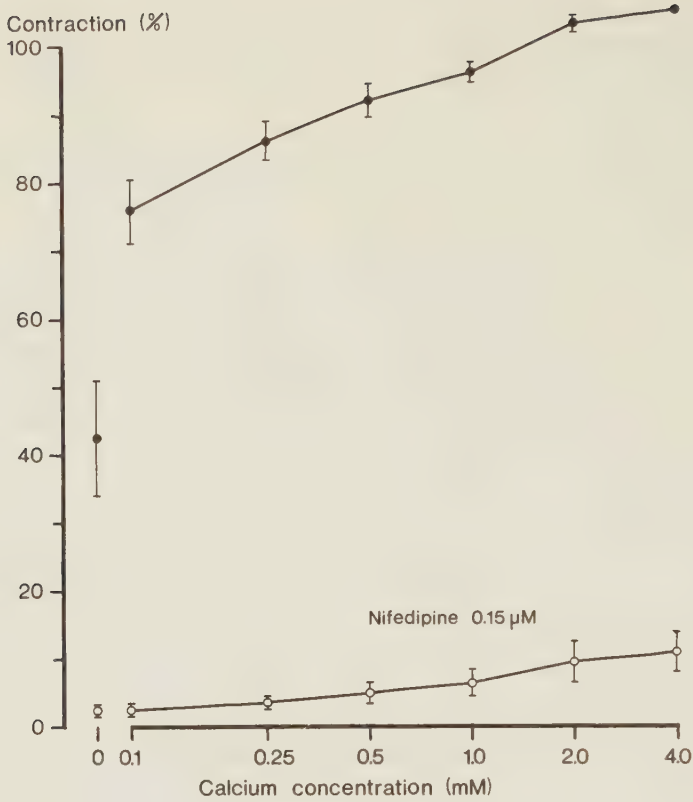


Fig.8 Responses of depolarized (127 mM K^+) human pial arteries to increasing concentrations of calcium in absence of drug (filled circles) and presence of nifedipine (open circles). Bars indicate mean values $\pm$ S.E.M.

cium concentration was increased from 0 to 4 mM (Fig. 8). In the absence of extracellular calcium pre-treatment with nifedipine for 10 min (and nimodipine in two experiments) caused a further reduction of the response to potassium from 43 ± 9 to 2.2 ± 0.2 per cent ($p < 0.001$) of the response in normal Krebs solution. Both nifedipine and nimodipine ($0.15 \mu\text{M}$) inhibited the contractions evoked when calcium was re-introduced into the organ bath (Fig. 8).

<u>Artery</u>	<u>Calcium antagonist</u>	<u>Potassium-induced contraction</u>			<u>PGF_{2α}-induced contraction</u>		
		N	EC ₅₀ (M)	Max. relax. (%)	N	EC ₅₀ (M)	Max. relax. (%)
Cerebral	Nifedipine	5	$(6.9 \pm 1.9) \times 10^{-9}$	94.8 $\pm$ 4.5	5	$(2.3 \pm 1.3) \times 10^{-8}$	59.1 $\pm$ 6.2
" "	Nimodipine	5	$(2.3 \pm 1.5) \times 10^{-9}$	94.4 $\pm$ 6.9	5	$(1.9 \pm 1.3) \times 10^{-8}$	65.5 $\pm$ 5.8
Mesenteric	Nifedipine	7	$(2.1 \pm 1.3) \times 10^{-8}$	96.8 $\pm$ 1.0	—	—	—
" "	Nimodipine	8	$(6.5 \pm 1.3) \times 10^{-9}$	96.2 $\pm$ 2.2	—	—	—

Table 1. Effects of nifedipine and nimodipine on potassium (127 mM) and PGF_{2α} (2.5 μM) induced contractions in pial arteries, and on potassium induced contractions in mesenteric arteries. Values given represent mean values $\pm$ S.E.M.

Discussion

It is well known that an increase in external potassium concentration within the range 1-15 mM can cause both relaxation and contraction of vascular smooth muscle (Haddy 1978). In the present study, no relaxant effect was observed when the potassium concentration was increased above 4.6 mM. The threshold for contraction was approximately 10 mM in cerebral arteries, and about 3-5 mM higher in mesenteric arteries. This difference between the two types of vessel may be attributed to differences in the electrogenic $\text{Na}^+ - \text{K}^+$ exchange. However, Casteels (1980) suggested that changes in extracellular potassium exert their action on the autonomic nervous system, and that changes of tension depend on the release of transmitter. If this suggestion is valid, the threshold difference may be ascribed to differences in the distribution of autonomic nerves in the arteries. On the other hand, the presence of cocaine and propranolol, or prazosin, did not change the threshold for potassium activation, which does not favour this view.

When contractions were induced by 127 mM potassium, maximum tension was obtained. Two phases were discernible in the contraction, one initial, rapid, and an ensuing more slowly developing. This pattern of response is well known from smooth muscle of other vascular regions, in animals as well as in man. The potassium contraction is probably dependent on extracellular or surface membrane associated calcium both for the rapid and the slow phase (van Breemen et al. 1972, Deth and van Breemen 1974). In agreement with this view, treatment in a calcium-free medium for 30 min rapidly and markedly decreased the responses to potassium. The reduction of NA and 5-HT induced contractions under the same conditions was conspicuously smaller. After re-introduction of calcium to the organ bath, the potassium-induced contractions were almost completely restored, in contrast to those induced by the amines. These findings are consonant with the view that the amines to some extent use intracellularly stored calcium for their contractile action and that these stores are relatively slowly refilled.

Drugs like nifedipine and nimodipine are believed to block the inflow of calcium through membrane potential sensitive calcium channels (Fleckenstein 1977, Bolton 1979). Supporting this concept, preparations contracted by potassium were almost completely relaxed by the calcium antagonists, whereas $\text{PGF}_{2\alpha}$ contracted cerebral arteries were relaxed only to approximately 60%. This agrees with findings in human crural arteries (Mikkelsen and Andersson 1978), and may be taken as evidence for an intracellular action of $\text{PGF}_{2\alpha}$. However, it might also be explained by $\text{PGF}_{2\alpha}$ opening calcium channels in the membrane that cannot be blocked by calcium antagonists. The finding that nifedipine only partly suppressed contractions induced by NA and 5-HT may be explained in a similar way.

After 30 min treatment in a calcium-free medium, the response to potassium (127 mM) of pial arteries was reduced by about 60%. A similar treatment of human crural arteries resulted in a 40% reduction of the potassium induced contraction (Mikkelsen et al. 1978). These findings suggest either that the time of exposure was insufficient to remove surface associated calcium, or that potassium also can mobilize intracellular calcium stores. Removal of calcium for 30 min. including pre-treatment with nifedipine for 10 min, almost completely reduced the response to potassium, a finding that may indicate that nifedipine not only prevents extracellular calcium from entering the cell, but also inhibits subsequent steps in the excitation-contraction coupling as suggested by Church and Zsotér (1980).

Shimizu et al (1980) found in experiments on dog cerebral and mesenteric arteries contracted by $\text{PGF}_{2\alpha}$, but not by potassium, that nifedipine had a greater relaxant effect on cerebral than on mesenteric vessels. The present findings suggest that nifedipine also in human vessels contracted by potassium was more effective in pial than in mesenteric arteries. This finding thus supports the concept of regional as well as species variations in sensitivity to calcium antagonists in vascular smooth muscle.

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EFFECTS ON FELINE CORTICAL PIAL MICROVASCULATURE OF TOPICAL
APPLICATION OF A CALCIUM ANTAGONIST (NIFEDIPINE) UNDER NORMAL
CONDITIONS AND IN FOCAL ISCHEMIA

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SUMMARY

Cat cortical arterioles and venules were exposed in vivo to the Ca^{++} antagonist nifedipine under normal conditions and in focal ischemia. Topical application of nifedipine caused a marked, concentration-dependent arteriolar dilatation. The dilatatory responses increased significantly with decreasing arteriolar size. Perivenular microapplication of nifedipine invariably caused dilatation which was less pronounced but more long-lasting than that on the arteriolar side. Arterioles constricted after middle cerebral artery (MCA) occlusion invariably dilated following nifedipine application. These dilatatory responses were transient but could be repeated, and on some occasions were accompanied by a return of flow in vessels in which stasis had been present. The results suggest that nifedipine is able to dilate cerebral vessels both under normal conditions and when contracted during focal ischemia.

INTRODUCTION

The Ca^{++} antagonist drug nifedipine induces relaxation of both isolated cerebral vessels in vitro (Allen GS & Banghart SB, 1979, Edvinsson L, Brandt L, Andersson et al., 1979) and feline cerebral arterioles in vivo after topical application (Brandt L, Andersson K-E, Bengtsson B, et al., 1979). The present study was designed to investigate in more detail the vascular responses of both the arteriolar and venular parts of the normal cortical pial microvasculature in vivo. It has also been demonstrated that topical application of nifedipine induces relaxation of feline cerebral arterioles in situ, which had been previously constricted by perivascular blood (Brandt L, Andersson K-E, Bengtsson B, et al., 1979) or cerebrospinal fluid (CSF) from patients with aneurysmal subarachnoid hemorrhage (SAH) (Brandt L, Ljunggren B, Andersson K-E, et al., 1980). In the present study we have also determined whether topical application of nifedipine could reverse the delayed arteriolar constriction which can occur in other pathological circumstances, namely focal cerebral ischemia (Teasdale G, Tamura A, Graham D, et al., 1981).

METHODS

The experiments were performed on 17 cats of either sex. After induction of anaesthesia with intravenous Saffan (alpha-xolone 6.75 mg/kg and alpha-dolone acetate 2.25 mg/kg b.wt.) and maintenance by alpha-chloralose (60 mg/kg) the intubated animals were ventilated with a respiratory pump with a rate and volume adjusted to maintain normoxia and normocapnia. End tidal pCO_2 as well as blood pressure were continuously monitored. Arterial blood samples were frequently checked for p_aCO_2 , p_aO_2 , pH and base deficit. Any base deficit was corrected with sodium bicarbonate given by slow intravenous infusion. Body temperature was kept at $37^\circ\text{C} \pm 0.5^\circ\text{C}$.

A left-sided craniectomy (3 x 2 cm) was performed with the head fixed in a stereotaxic frame and the dura reflected. The exposed gyri were irrigated continuously with mineral oil maintained at a temperature of 37°C .

Pial vessel caliber was measured by a television image-splitting technique (MacKenzie ET, Strandgaard S, Graham DI, et al., 1976). Perivascular microinjections were made by the use of micropipettes with a tip diameter of approximately 8 μm . To minimize any change in pH of the solutions in the pipettes, the tops were sealed with mineral oil and the tips submerged in the bulk of the solution and sealed under oil (Harper AM & MacKenzie ET, 1977). A hydraulic syringe was used to inject approximately 2 μl of the solution into the perivascular space.

Nifedipine obtained from Bayer AG, dissolved in aethanol, polyethylene glycol, and water, was further diluted in a mock cerebrospinal fluid solution (for composition, see Ref, 5). The final pH of the solution was 7.18. Nifedipine in 5 different concentrations (0.02, 0.2, 2.0, 20.0 and 40.0 $\mu\text{g/ml}$) was applied to the arterioles whereas the venules were exposed to the 20.0 $\mu\text{g/ml}$ concentration. Test solutions containing the aethanol and polyethylene glycol vehicle corresponding to the different nifedipine solutions were prepared. To prevent decomposition of nifedipine the solutions were protected from light exposure except during perivascular injections.

Focal ischemia in the parietal cortex was induced by clamping the left middle cerebral artery (MCA) through a transorbital exposure. For description of pial vessel caliber changes following MCA occlusion see Teasdale G, Tamura A, Graham D, et al., 1981.

RESULTS

The resting diameters of the arterioles that were studied were in the range 34 to 246 μm and of the venules 41 to 216 μm . All perivascular microinjections were performed at normocapnia (5.1 ± 0.5 kPa) and normotension (116.1 ± 7.3 mm Hg (mean $\pm$ SD).

Arteriolar responses

Topical application of nifedipine caused a concentration-dependent arteriolar dilatation (fig.1). The dilatatory responses were prompt with peak values mostly reached within 90 sec. The dilatatory response increased significantly ($p < 0.001$)

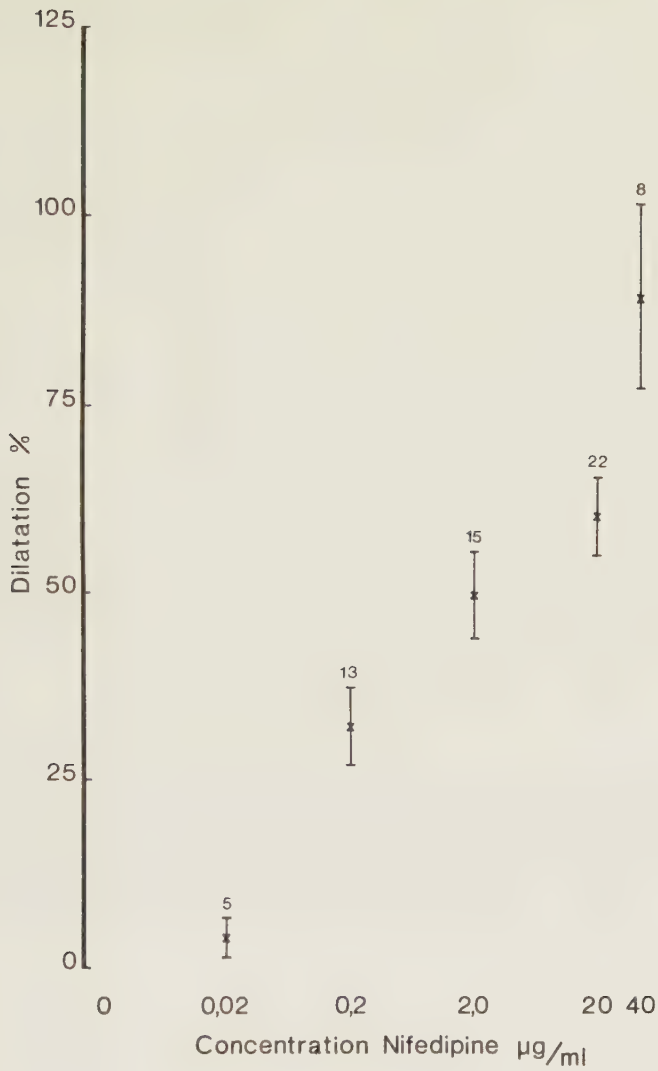


Fig. 1 Arteriolar dilatatory responses to 5 different concentrations of nifedipine. The values are expressed as a percentage increase from resting vessel caliber. The bars indicate mean values $\pm$ S.E.M.

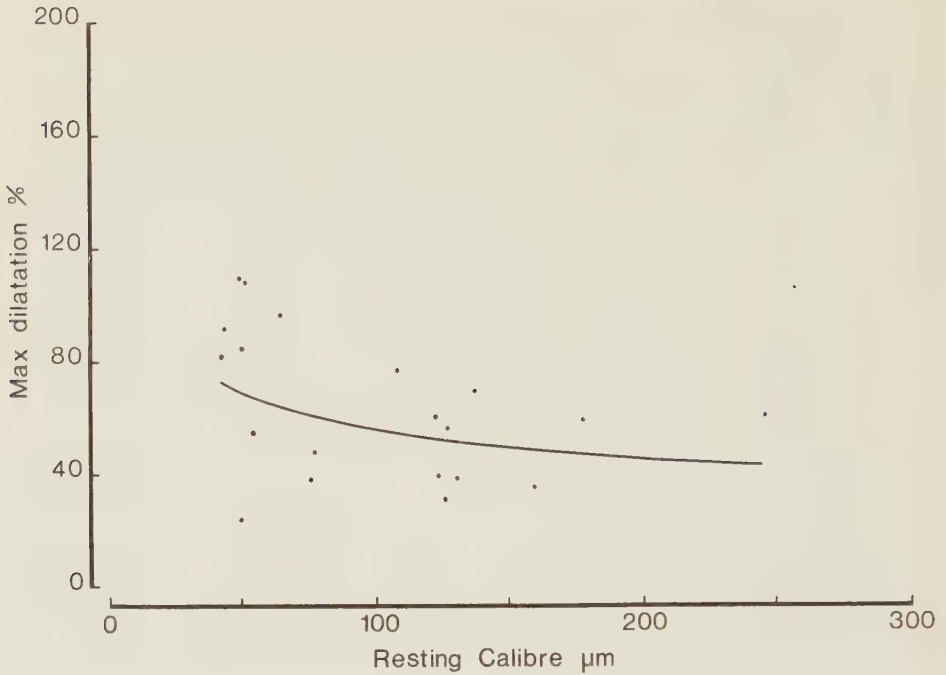


Fig. 2 The individual arteriolar dilatatory responses to nifedipine (20 µg/ml) expressed as percentage deviation from resting vessel caliber. Note the more marked dilatation with decreasing vessel caliber. The slope of the regression line is significantly different from zero ($p < 0.001$). Equation for the regression line: $Y = A \cdot x^B$.

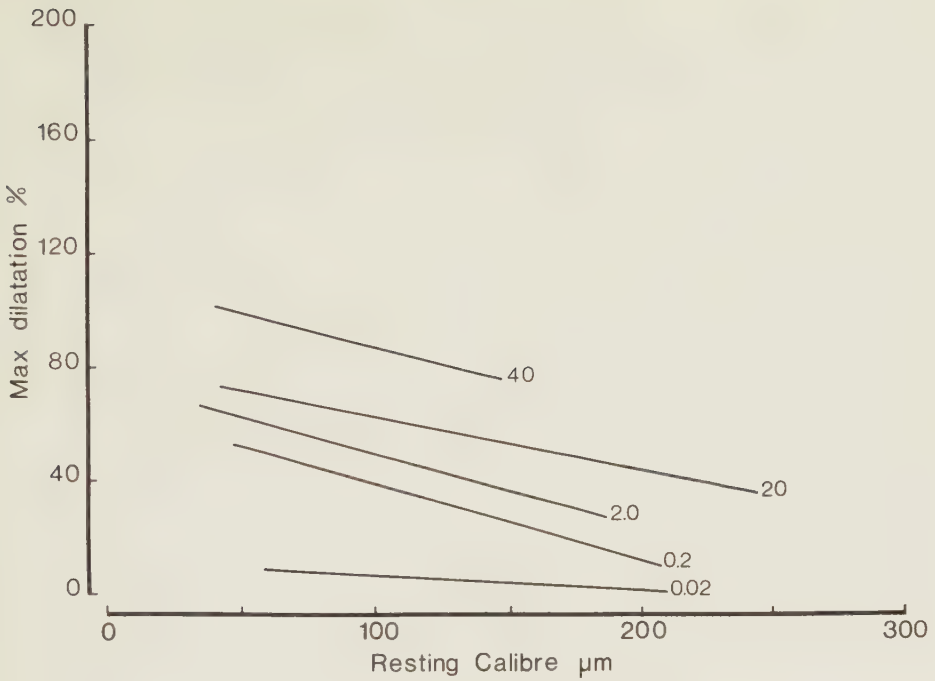


Fig. 3 Linear regression lines for the dilatatory effects of nifedipine in 5 different concentrations. Equations for the regression lines: $Y = A + B \cdot x$. The concentration values ($\mu\text{g/ml}$) for the different nifedipine solutions are given in the figure.

with decreasing arteriolar size. The individual dilatatory responses to one nifedipine concentration (20 ug/ml) are shown in fig. 2. The linear regression of the dilatatory responses to each nifedipine concentration are given in fig. 3. The arterioles returned to resting diameter within 10-15 minutes after the nifedipine applications.

The arteriolar dilatatory responses to vehicle solutions corresponding to 20.0 ug/ml and 40.0 ug/ml nifedipine solutions are illustrated in Fig.4. As shown mock CSF elicited a slight and transient dilatatory response closely resembling that after application of the vehicle solution corresponding to 20.0 ug/ml nifedipine. By contrast, after application of the vehicle solution corresponding to 40.0 ug/ml of nifedipine, the dilatation was slower in onset but longer in duration and more pronounced.

Venular responses

The application of nifedipine around 16 venules invariably resulted in a dilatation which ranged from 16.2 to 47.9% (mean value 26.1%). The responses of these veins were less pronounced than those of arterioles of comparable size (Fig.5). When compared with the effect of vehicle solution on veins (Fig.6) the effects of nifedipine were of a more gradual onset, building up during 2 to 6 minutes - and more persistent. Maximal nifedipine-induced dilatatory responses were $26 \pm 4\%$ as compared to maximal vehicle-induced dilatatory responses of $15 \pm 2\%$. The venules returned to pre-injection diameter within approximately 15 minutes after vehicle-injections, whereas after nifedipine, the effect persisted for 20 minutes (fig.6).

Arteriolar responses in ischemic cortex

After MCA occlusion, all pial arterioles showed a dilatation which first became apparent 15-30 seconds after occlusion. All vessels in the antero-lateral gyrus showed sustained dilatation whereas delayed constriction occurred in the suprasylvian gyrus and most frequently in the ecto-sylvian gyrus 2-9 minutes after occlusion (Teasdale G, Tamura A, Graham D, et al., 1981).

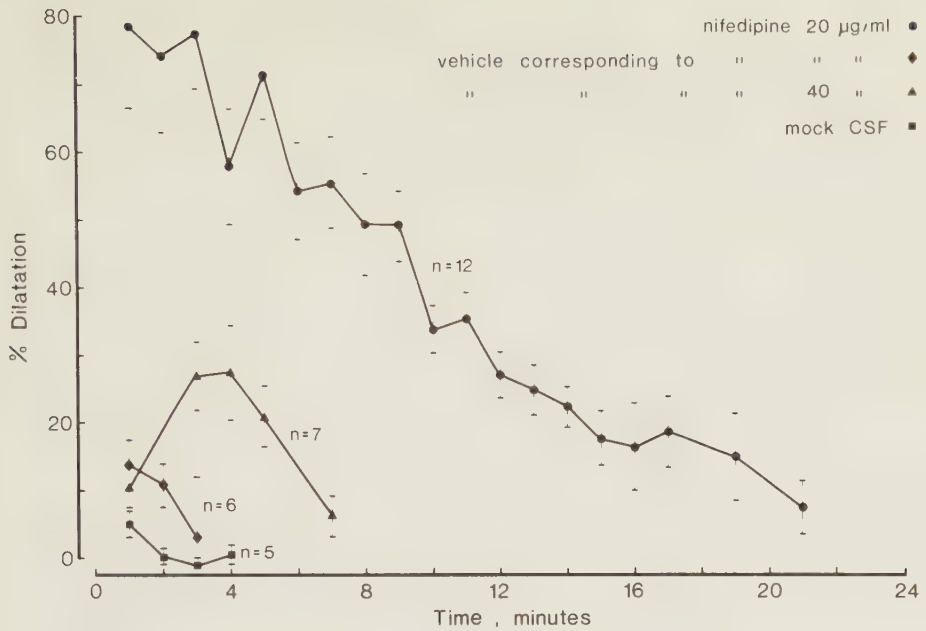


Fig. 4 Time courses for dilatatory effects of nifedipine (20 µg/ml), vehicle corresponding to 20 and 40 µg/ml nifedipine solutions, respectively, and mock CSF. The arteriolar responses are expressed as percentage deviation from resting vessel caliber. Bars indicate mean values $\pm$ S.E.M.

Fig. 5 Comparison between venular and arteriolar dilatatory responses to nifedipine(20 $\mu\text{g/ml}$), corresponding vehicle, and mock CSF, respectively. Bars indicate mean values $\pm$ S.E.M.

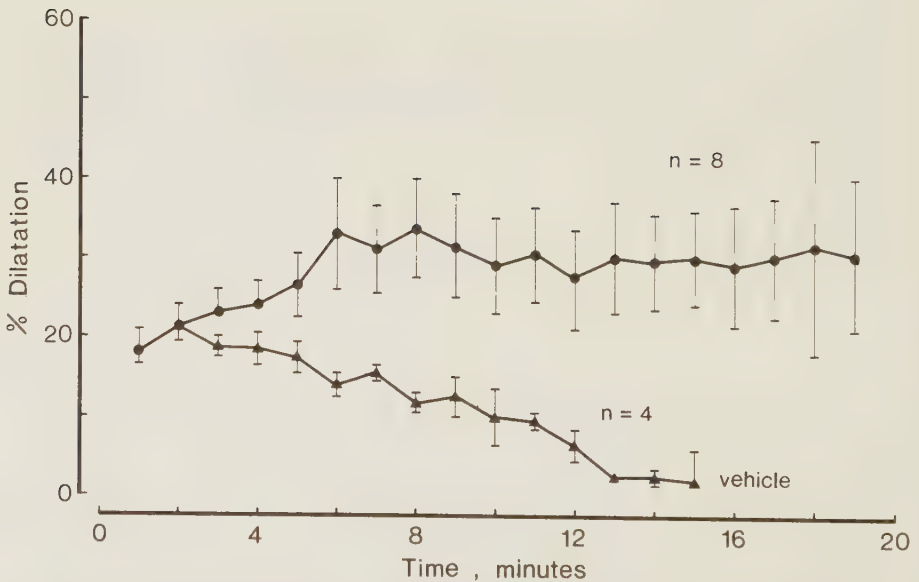
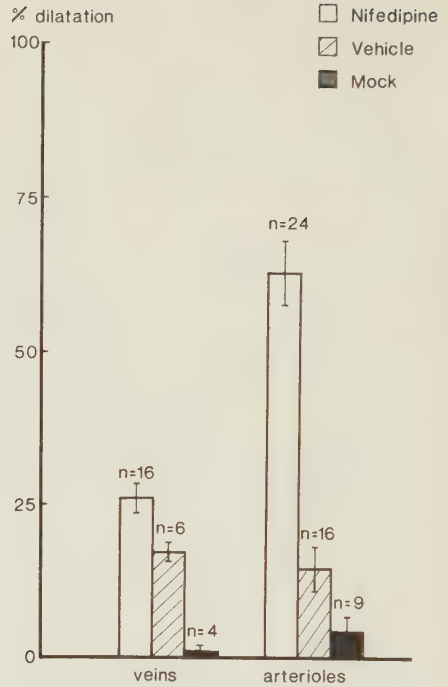


Fig. 6 Time course for the venular dilatation induced by nifedipine (20 $\mu\text{g/ml}$, filled circles) and corresponding vehicle. Bars indicate mean values $\pm$ S.E.M.

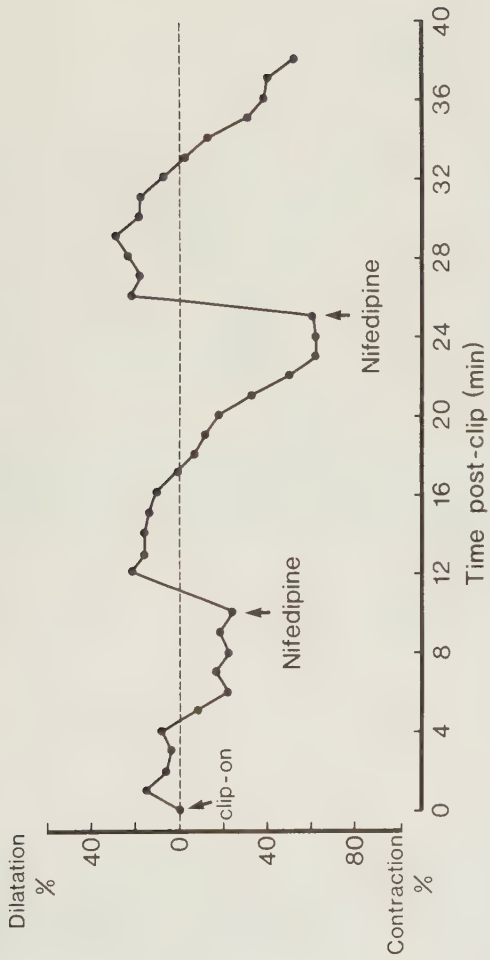


Fig. 7 Time course of the caliber changes of an arteriole in the ectosylvian gyrus after MCA occlusion and repeated perivascular microapplication of nifedipine ($20 \mu\text{g/ml}$). The resting vessel caliber before MCA clipping was $56.3 \mu\text{m}$.

When nifedipine 20.0 ug/ml was injected around the arterioles that had become constricted, there was invariably a marked arteriolar dilatatory response (fig.7). These dilatatory responses were transient but could be repeated (fig.7), and on some occasions were accompanied by a return of flow in vessels in which stasis had been present. (Figs. 8a and b).

DISCUSSION

Smooth muscle tone is thought to be maintained by release of intracellular calcium (Rüegg JC, 1971). Calcium initiates contraction by activating the enzyme myosin-light-chain kinase which causes phosphorylation of myosin, which is necessary for its interaction with actin (Small JV & Sobleszek A, 1977). Golenhofen & Helmstein (1973) have suggested the existence of two main types of calcium-dependent activation mechanisms in smooth muscle: one correlated to spike discharges and responsible for phasic activation (P-mechanism) and the other correlated to a spikefree depolarization and responsible for tonic activation (T-mechanism).

It cannot be excluded that calcium influx during these types of activation occurs through separate channels in the membrane (Bolton, 1979). According to Fleckenstein (1977) calcium antagonistic drugs selectively inhibit the calcium influx into the cell and the main site of their action is the slow channels in the membrane. Different calcium antagonists are said to block the P-mechanism more or less selectively, D600 and nifedipine showing the highest selectivity (Golenhofen & Hermstein, 1975). These drugs also block preferably the voltage sensitive calcium channels possibly associated with phasic activation (Bolton, 1979).

The present study clearly demonstrates that nifedipine when locally applied around cat pial vessels in situ results in a consistent dose-related dilatation. The consistent pattern of increasing dilatatory responses with decreasing vessel size expressed as percentage vessel diameter change from resting vessel caliber is in agreement with the observations of Betz and Csornai (1978), who performed microperfusions of the perivascular space in cats with a calcium-free mock CSF also con-



a.



b.

Fig. 8 An arteriole in the ecto-sylvian gyrus 20 min. post-MCA occlusion before (a) and 45 sec. after (b) perivascular microapplication of nifedipine ($20 \mu\text{g/ml}$). In the small branches stasis was present (a); return in flow was noted during dilatation (b). The dark line across the picture (b) represents an optic artefact.

taining EGTA. A possible explanation for the increasing arteriolar reaction with decreasing vessel diameter might be that the tone of small arterioles is not only higher, but also more dependent on extracellular calcium, thus making smaller vessels more sensitive to a blockade of accessible extracellular calcium ions. This in turn suggests that the phasic activation (P-mechanism) is of greater importance as compared to tonic activation (T-mechanism) in the smaller vessels. Consequently, calcium antagonists can be expected to have a more pronounced effect on smaller vessels.

In the present study a step-wise increase in the concentrations of the applied nifedipine solutions caused a corresponding step-wise increase in dilatatory response. However, increasing the nifedipine concentration from 20.0 ug/ml to 40.0 ug/ml caused an unexpected exaggeration of the dilatatory response. As illustrated in fig.4 the vehicle, in a concentration corresponding to that used with 20.0 ug/ml nifedipine, caused only a small and transient dilatatory response while that of a concentration corresponding to the 40.0 ug/ml nifedipine solution induced a much more pronounced and also more longlasting dilatatory response. Hence, the very pronounced dilatatory effect of the 40.0 ug/ml solution might represent the combined effects of the drug and vehicle.

The dilatatory responses to nifedipine reached its maximum within approximately 60 sec and were invariably more longlasting (> 10 min) than those observed by other investigators following perivascular application of 5-hydroxytryptamine (5-HT, serotonin) to cat pial arterioles with a resting caliber < 70 um. The duration of the 5-HT effects were less than 120 sec (Harper AM & MacKenzie ET, 1977), probably reflecting effective mechanisms in ^{de}activation or elimination of the amine. The more longlasting effect of nifedipine might be because its high lipid solubility slows its removal from the vessel wall.

Microapplication of the mock CSF invariably caused a minor and transient dilatation, which is in agreement with the results of other investigators using the same model, though on spinal pial vessels (Crawford RA, Gregory PC & Griffiths IR, 1980).

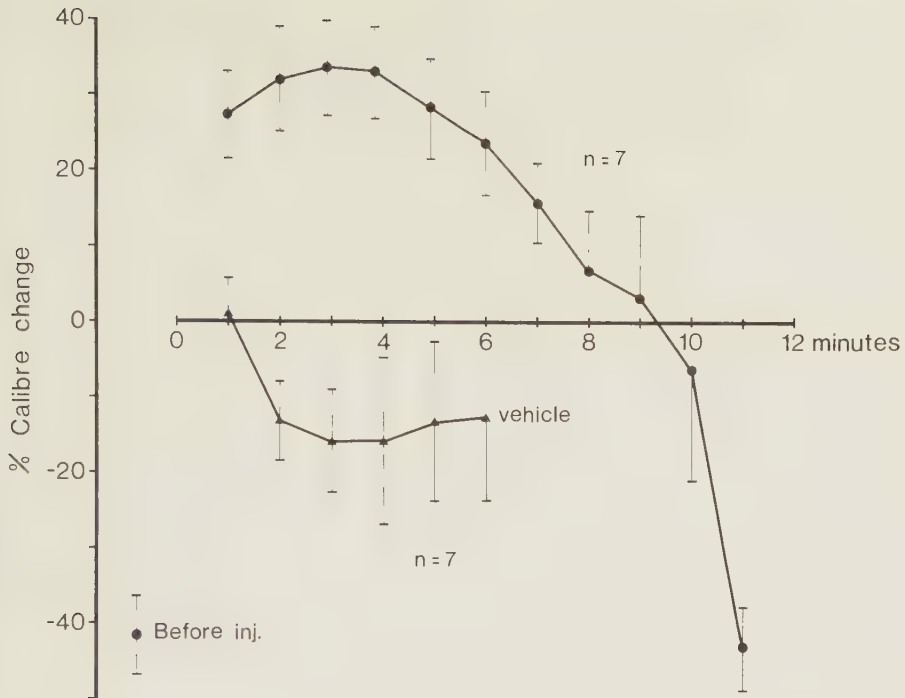


Fig. 9 Dilatatory responses of 'ischemic' arterioles after microapplication of nifedipine ($20 \mu\text{g/ml}$) and the corresponding vehicle. Bars indicate mean values $\pm$ S.E.M.

The dilatatory effect of nifedipine on the venular part of the pial microvasculature indicates that cerebral venules have a tone which is dependent on extracellular calcium. The observation that the venular dilatatory responses were less pronounced than the arteriolar responses could probably be explained by less elasticity of the venular wall and/or a lower intravascular pressure. A striking difference in the time-course between venular dilatations versus arteriolar dilatations was observed. This difference does not favour the concept that venular dilatations are simply due to a passive pressure-effect, because of increased blood flow secondary to dilatations of adjacent arterioles.

The difference in the dilatatory responses between arterioles and venules is illustrated in fig.5 which also shows the differences in responses to the vehicle alone. Although the maximum responses to the nifedipine and vehicle solutions did not differ significantly on the venules, there was a striking difference in the time-course of the ensuing vasodilatation in the two types of induced dilatation, indicating different modes of action. (Fig.6).

In the series of experiments where focal ischemia was produced and nifedipine was applied to arterioles showing delayed constriction, this was invariably and immediately reversed. (Fig.9). The resulting dilatation indicates that the mechanisms behind the delayed ischemic arteriolar constriction is calcium-dependent. A comparison between mean arteriolar dilatatory responses to 20.0 ug/ml nifedipine on normal vessels and constricted vessels in focal ischemia is given in fig.10. In the 'ischemic' arterioles the amplitude of nifedipine-induced dilatations from maximum constriction level does not differ significantly from the amplitude of the dilatations of normal arterioles from resting vessel caliber. However, when maximum dilatation from resting vessel caliber of both groups is compared an interesting difference between the 'ischemic' vessels and normal vessels is apparent. If this difference is not a consequence of decreased intraluminal pressure in 'ischemic' arterioles, then the constrictions observed during ischemia, seem to be elicited at least partly via mechanisms insen-

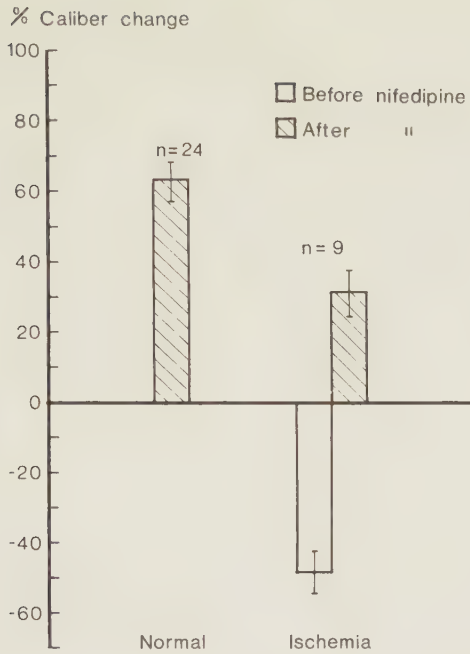


Fig. 10 Comparison between the dilatatory responses to nifedipine (20 μ g/ml) in normal and 'ischemic' arterioles. Bars indicate mean values $\pm$ S.E.M.

sitive to calcium inhibitors of the nifedipine type. If nifedipine acts only by inhibiting calcium influx through membrane potential sensitive calcium channels the present findings indicate that arteriolar constriction during ischemia is partly caused either by influx of calcium through calcium channels of another type or by release of calcium from intracellular stores. Church & Zsoter (1980) suggested that nifedipine also has an intracellular site of action inhibiting the release of calcium. Whether such a mechanism is of importance for the relaxant effect shown in the present experiments is an open question.

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A presentation of the preliminary results of the present study was given at the Satellite Symposium of the XXVIII International Congress of Physiological Sciences in Tübingen, West-Germany July 22-25, 1980 and at the 2nd Beaune conference on acute cerebrovascular diseases & pathopharmacology, Beaune, France October 27-28, 1980.

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